

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY AMENDMENT TO NDA REVIEW

Application number: 205123
Supporting document/s: 1
Applicant's letter date: 3/28/2013
CDER stamp date: 3/28/2013
Product: Simeprevir
Indication: Treatment of chronic hepatitis C (genotype I)
Applicant: Janssen Research and Development LLC
Review Division: Division of Anti-viral Products
Reviewer: Janice Lansita, PhD, DABT
Supervisor/Team Leader: Hanan Ghantous, PhD, DABT
Division Director: Debra Birnkrant, MD
Project Manager: Victoria Tyson

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Pilot Mouse Embryofetal Study Exencephaly Findings: Human Relevance and Labeling

Overview: This amendment to the original NDA review serves to further describe the scientific rationale for not including the pilot mouse embryofetal exencephaly finding in labeling.

Review: Teratogenicity was observed in a TMC435 pilot mouse embryofetal study¹ at doses up to 2000 mg/kg. The teratogenic findings were exencephaly and protruding tongue in 6/50 fetuses at 1000 mg/kg (from 2 out of 4 litters) and 4/64 fetuses at 2000 mg/kg (from 2 out of 6 litters). The teratogenic findings were not reproduced in the pivotal mouse embryofetal study². In this study, the dose volume was reduced from 10 ml/kg to 5 ml/kg and the high dose was set at 1000 mg/kg, based on the sponsor's determination of the, "maximum recommended dosing volume of 5 mL/kg with PEG400 and maximum feasible concentration of 200 mg/mL...." The pilot study findings occurred at a higher incidence than the historical control data (Janssen R&D Test Facility, Beerse site) with no cases in 0/815 fetuses, of either exencephaly or protruding tongue reported from a total of 6 mouse embryofetal studies conducted between 2007-2013. These data are consistent with the historical control data for the pivotal study (b) (4): exencephaly was observed in 2/1950 (0.1%) fetuses and protruding tongue was observed in 1/1950 (0.05%) fetuses from a total of 10 studies from 2003-2011.

TMC435 findings in the pivotal mouse embryofetal study included two maternal deaths at 1000 mg/kg, high post-implantation loss, decreased fetal weight and an increase in skeletal variations. **Although test article related teratogenic findings were not observed, exencephaly was seen in 1/260 control fetuses, 1/253 fetuses at the low dose, and 1/234 fetuses at the high dose; because exencephaly was seen in both control and treated groups at a low incidence, the finding was not attributable to treatment.** Teratogenicity was not observed in the rat embryofetal toxicity study however, TMC435 exposures were lower in the rat than in the mouse.

In this reviewer's opinion, it is not clear that the exencephaly and protruding tongue findings were related to TMC435. The following differences may explain why the teratogenic findings were not reproduced: 1) the pivotal study was conducted at a different laboratory than the pilot study, 2) the mice were from a different source, 3) the testing conditions were different between the two laboratories and finally, 4) the dose volume was reduced which could correlate with reduced maternal toxicity or maternal stress.

The test article relationship of the exencephaly and protruding tongue findings in the non-GLP pilot study is questionable because the findings were observed with a low N-value of 6 pregnant mice per group at an incidence of 6-12% in the mouse fetus. Based on the pilot study incidence, at a higher N-value of 24 pregnant mice per group in the

pivotal study, one would expect 15-28 fetuses with exencephaly/protruding tongue out of 234 total fetuses at the high dose which was not observed.

In mice, exencephaly can be caused by maternal toxicity or stress as reported in the literature. Reported maternal stressors associated with exencephaly in the mouse include high frequency noise, physical restraint/handling, and maternal toxicity^{3 4 5 6 7}. Additionally, physical restraint or immobilization has been associated with increased levels of the stress hormone cortisol in mice⁸. Of the potential stressors that could contribute to the exencephaly finding, the most probable would include physical restraint/handling as well as maternal toxicity.

The nonclinical formulation of TMC435 is PEG-based, highly viscous, and difficult to administer by the oral gavage route at high doses⁹. Several early deaths and clinical signs/macrosopic findings (salivation, chin rubbing, vomiting, audible respiration, abdominal distension, abnormal contents in the GI, inflammation of the respiratory tract) were seen across the nonclinical studies and attributed to the gavage procedure/viscous formulation. Most notably in the 6-month rat toxicity study with 15/240 early deaths attributed to the gavage procedure^{10 11}. Therefore, more stress may have been put on the maternal mice administered the higher 10 ml/kg dose volume possibly resulting in exencephaly in the mouse fetus.

Another possibility is that the difference in dose volume may have exacerbated a delay in gastric emptying or GI toxicity. Delayed gastric emptying and GI toxicity could potentially result in maternal toxicity as well as nutritional deficits in the maternal and fetal animals. In the 3-month mouse oral gavage study, 20/188 early deaths were attributed to a delay in gastric emptying resulting in inflammation of the respiratory tract following aspiration of the viscous test article. Dose volume appeared to play an important factor in the early deaths (many within the first week of dosing) associated with a 10 ml/kg dose volume (doses up to 2000 mg/kg). Upon a reduction of the dose volume to 5 ml/kg on Day 8 for the high dose (top dose lowered to 1000 mg/kg) and Day 9 for all other dose groups, the incidence of early mortality appeared to be reduced¹¹. The stomach capacity of the mouse is approximately 0.4 mls¹². At a higher dose volume, the volume administered to achieve 1000 and 2000 mg/kg would be 0.25 mls or 63% of the stomach capacity in mice. At the lower dose volume, the volume administered to achieve 1000 mg/kg would be 0.125 mls or 32% of the stomach capacity. In both the pilot and pivotal mouse embryofetal studies, GI toxicity (distention and watery or abnormal contents in the GI tract) was observed in 1 out of 2 of the early maternal deaths in each study. A transient decrease in maternal food consumption (Day 6-11) and a decrease in fetal body weight at 2000 mg/kg were seen in the pilot study with no clear impact on maternal body weight or litter survival. In the pivotal study, the maternal early deaths showed significant decreases in maternal body weight and complete litter resorptions. Additionally, fetal body weight was decreased at 1000 mg/kg. Maternal toxicity was seen in both the pilot and pivotal studies with a higher rate of early mortality in the pilot study (2/6, 33%) compared with the pivotal study (2/24, 8.3%). Maternal GI toxicity may have contributed to the exencephaly findings.

Conclusion: In conclusion, there is no clear teratogenic signal with TMC435. The exencephaly findings were not reproduced in the pivotal study and may have been a secondary effect due to maternal stress or toxicity. It is this reviewer's recommendation to not include the exencephaly and protruding tongue findings in labeling.

References:

1. Study NC187: Pilot oral developmental toxicity study of TMC435350 in the mouse.
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8. Miller, DB and Chernoff, N. Restraint-induced stress in pregnant mice — Degree of immobilization affects maternal indices of stress and developmental outcome in offspring. *Toxicology*. 1995; 98(1–3):177-186.
9. NC257: 2-week Repeated Dose Oral Toxicity Study of TMC435350 in the Rat.
10. NC179: 6-mos Repeated Dose Oral Toxicity Study of TMC435350 in Rat w/ 3 mos interim kill.
11. NC206: 3-Month Repeated Dose Oral Toxicity Study of TMC435350 in the Mouse.
12. McConnell, E. L., Basit, A. W. and Murdan, S. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. *Journal of Pharmacy and Pharmacology*. 2008; 60: 63–70.

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/s/

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11/18/2013

HANAN N GHANTOUS
11/18/2013

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1 EXECUTIVE SUMMARY

1.1 INTRODUCTION

TMC435 (simeprevir) is a HCV NS3/4A protease inhibitor for the treatment of chronic HCV infection (genotype 1) in combination with peginterferon alfa and ribavirin, in adults with compensated liver disease (including cirrhosis) who are treatment-naïve or who have failed previous interferon therapy (pegylated or non-pegylated) with or without ribavirin. The proposed dose regimen is one 150 mg capsule taken orally once daily with food for 12 weeks in combination with peginterferon alfa and ribavirin. The drug product is an immediate-release hard gelatin capsule (b) (4) (b) (4) equivalent to 150 mg of drug substance (simeprevir (b) (4) (b) (4)).

The nonclinical safety package for TMC435 was adequate and included pharmacology, safety pharmacology, ADME, general toxicology, reproductive toxicology, and genotoxicity studies. Carcinogenicity studies were not required based on the 12 week indication and negative genotoxicity studies. Nonclinical studies were conducted across various species. Embryofetal studies were conducted in the mouse and rat because the rabbit had poor bioavailability (2.5%). The rat and dog were evaluated in the pivotal toxicology studies and appeared to be relevant species based on overall similar PK and metabolite profiles compared with human. The Na-salt of TMC435 was also tested via the dietary route in mice and rats, in order to increase exposures and to avoid mortality caused by entry of the irritant and viscous formulation into the respiratory tract upon administration via oral gavage.

1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS

The major target organs identified in the TMC435 nonclinical studies include the gastrointestinal tract (vacuolation of apical enterocytes, dilatation of lacteals) and the liver (hepatocellular necrosis, centrilobular hypertrophy, increases in ALT, AST, ALP, and bilirubin), consistent with the clinical trial safety profile. There are no safety margins for these toxicities. The liver and GI have been identified as target organs in clinical trials. The heart was identified to be a potential target organ (acute endocardial and myocardial necrosis) in the dog at high doses (~28X the clinical AUC); no cardiac safety signals have been identified in the clinical trials.

Potential reproductive toxicity effects in the pregnant rat and mouse (mortality and post-implantation loss), the fetus (skeletal variations, kinked tail, adverse body weight decrease) as well as the developing offspring (adverse body weight decrease, small size, motor activity decreases) were observed with no exposure multiples in the rat and a 4X multiple in the mouse for the reproductive toxicities. The potential reproductive toxicity risks will be mitigated by appropriate labeling. Currently, use of TMC435 is to be in combination with ribavirin and peginterferon alpha which have known fertility and teratogenicity risks and are contraindicated in pregnancy; therefore, the potential for TMC435 to have an additional impact on pregnancy risk is currently low. However, if TMC435 is used without the combination of ribavirin and peginterferon alpha, the potential risk of TMC435 on pregnancy and the developing fetus/offspring may be critical.

Although there are no to low exposure multiples for the identified nonclinical toxicities over the anticipated human exposure, the identified risks appear to be acceptable. In conclusion, there are no significant issues that would preclude the approval of simeprevir (TMC435) from a nonclinical perspective.

1.3 RECOMMENDATIONS

1.3.1 Approvability

Yes.

1.3.2 Additional Non Clinical Recommendations

1.3.3 Labeling

SPONSOR'S PROPOSED LABELING

8.1 PREGNANCY

(b) (4)

Pregnancy Category X: Use with Ribavirin and Peginterferon Alfa

Animal studies have shown that ribavirin causes birth defects and/or fetal deaths while peginterferon alfa is abortifacient [see *Contraindications (4)* and *Warnings and Precautions (5.2)*]. See the prescribing information for ribavirin. Significant teratogenic and/or embryocidal effects have been demonstrated in all animal species exposed to ribavirin; and therefore ribavirin is contraindicated in women who are pregnant and in the male partners of women who are pregnant [see *Contraindications (4)*, *Warnings and Precautions (5.2)* and ribavirin prescribing information]. Interferons have abortifacient effects in animals and should be assumed to have abortifacient potential in humans [see peginterferon alfa prescribing information].

Extreme caution must be taken to avoid pregnancy in female patients and female partners of male patients while taking this combination. Women of childbearing potential and their male partners should not receive ribavirin unless they are using effective contraception (two reliable forms) during treatment with ribavirin and for 6 months after treatment.

A Ribavirin Pregnancy Registry has been established to monitor maternal-fetal outcomes of pregnancies in female patients and female partners of male patients exposed to ribavirin during treatment and for 6 months following cessation of treatment. Health care providers and patients are encouraged to report such cases by calling 1-800-593-2214.

13 NONCLINICAL TOXICOLOGY

13.1 CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Carcinogenesis and Mutagenesis

Use with Ribavirin and Peginterferon alfa: Ribavirin is genotoxic in *in vitro* and *in vivo* assays. Ribavirin was not oncogenic in a 6-month p53+/- transgenic mouse study or a 2-year carcinogenicity study in rat. See the prescribing information for ribavirin.

Simeprevir was not genotoxic in a series of *in vitro* and *in vivo* tests. Carcinogenicity studies with simeprevir have not been conducted.

Fertility

Use with Ribavirin and Peginterferon alfa: Animal studies have shown that ribavirin induced reversible toxicity in males while peginterferon alfa may impair female fertility. See the prescribing information for ribavirin and peginterferon alfa.

(b) (4)

REVIEWER'S DRAFT CHANGES TO SPONSOR'S PROPOSED LABELING

8.1 PREGNANCY

Pregnancy Category X: Use with Ribavirin and Peginterferon Alfa

Extreme caution must be taken to avoid pregnancy in female patients and female partners of male patients while taking this combination. Women of childbearing potential and their male partners should not receive ribavirin unless they are using effective contraception (two reliable forms) during treatment with ribavirin and for 6 months after treatment.

A Ribavirin Pregnancy Registry has been established to monitor maternal-fetal outcomes of pregnancies in female patients and female partners of male patients exposed to ribavirin during treatment and for 6 months following cessation of treatment. Health care providers and patients are encouraged to report such cases by calling 1-800-593-2214.

Animal Data

Animal studies have shown that ribavirin causes birth defects and/or fetal deaths while peginterferon alfa is abortifacient [see *Contraindications (4)* and *Warnings and Precautions (5.2)*]. See the prescribing information for ribavirin. Significant teratogenic and/or embryocidal effects have been demonstrated in all animal species exposed to ribavirin; and therefore ribavirin is contraindicated in women who are pregnant and in the male partners of women who are pregnant [see *Contraindications (4)*, *Warnings and Precautions (5.2)* and ribavirin prescribing information]. Interferons have abortifacient effects in animals and should be assumed to have abortifacient potential in humans [see peginterferon alfa prescribing information].

Pregnancy Category C: TRADENAME

There are no adequate and well-controlled studies with TRADENAME alone or in combination with peginterferon alfa and ribavirin in pregnant women.

Animal Data

In a mouse embryofetal study at doses up to 1000 mg/kg, simeprevir resulted in early death and spontaneous abortions in pregnant mice at an exposure approximately 6 times higher than the AUC in humans at the recommended 150 mg daily dose. Significantly decreased fetal weights and an increase in fetal skeletal variations were seen at exposures approximately 4 times higher than the AUC in humans at the recommended daily dose.

(b) (4)

In a rat peri- post-natal study, maternal animals were exposed to simeprevir during gestation and lactation at doses up to 1000 mg/kg/day. In pregnant rats, simeprevir resulted in early deaths

(b) (4)

The developing rat offspring exhibited significant decreased body weight, small size, and negative effects on physical development (decreased motor activity (b) (4)) following simeprevir exposure in utero (via maternal dosing) and during lactation (via maternal milk to nursing pups) at a maternal exposure similar to the (b) (4) AUC at the recommended 150 mg once daily dose.

13 NONCLINICAL TOXICOLOGY

13.1 CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Carcinogenesis and Mutagenesis

Use with Ribavirin and Peginterferon alfa: Ribavirin is genotoxic in *in vitro* and *in vivo* assays. Ribavirin was not oncogenic in a 6-month p53+/- transgenic mouse study or a 2-year carcinogenicity study in rat. See the prescribing information for ribavirin.

Simeprevir was not genotoxic (b) (4) in a series of *in vitro* and *in vivo* tests including the Ames test, the mammalian forward mutation assay in mouse lymphoma cells or the *in vivo* mammalian micronucleus test. Carcinogenicity studies with simeprevir have not been conducted.

Fertility

Use with Ribavirin and Peginterferon alfa: Animal studies have shown that ribavirin induced reversible toxicity in males while peginterferon alfa may impair female fertility. See the prescribing information for ribavirin and peginterferon alfa.

(b) (4)

13.2 Animal Toxicology and/or Pharmacology

Cardiovascular toxicity consisting of acute endocardial and myocardial necrosis restricted to the left ventricular subendocardial area was seen in a 2-week oral dog toxicity study at an exposure approximately 28 times the AUC in humans at the recommended daily dose of 150 mg.

2 DRUG INFORMATION

2.1 DRUG

The drug product is an immediate-release hard gelatin capsule for oral administration, containing 154.6 mg of (b) (4) (simeprevir sodium salt), which is equivalent to 150 mg of drug substance (simeprevir (b) (4)).

The container closure system is an (b) (4) high density polyethylene bottle (b) (4).

Generic Name

simeprevir

Code Name

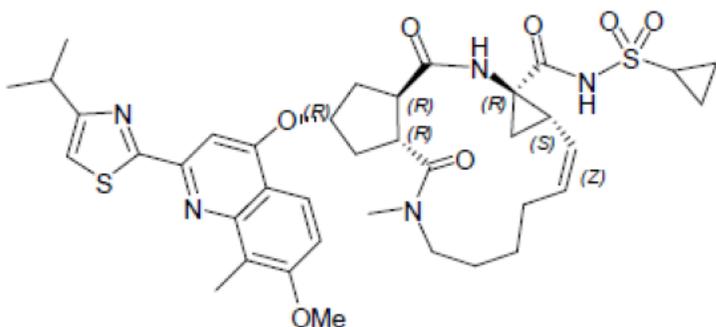
TMC435, R494617, or JNJ-38733214-AAA

Chemical Name

(2*R*,3*aR*,10*Z*,11*aS*,12*aR*,14*aR*)-*N*-(cyclopropylsulfonyl)-2-[[2-(4-isopropyl-1,3-thiazol-2-yl)-7-methoxy-8-methyl-4-quinolinyl]oxy]-5-methyl-4,14-dioxo-2,3,3*a*,4,5,6,7,8,9,11*a*,12,13,14,14*a*-tetradecahydrocyclopenta[*c*]cyclopropa[*g*][1,6]diazacyclotetradecine-12*a*(1*H*) carboxamide

Molecular Formula/Molecular Weight

C₃₈H₄₇N₅O₇S₂ /MW 749.94

Structure or Biochemical Description**Pharmacologic Class**

Inhibitor of HCV NS3/4A protease for the treatment of chronic HCV infection (genotype 1).

2.2 RELEVANT INDS, NDAS, BLAS AND DMFS

IND 75391

2.3 DRUG FORMULATION**Clinical formulation**

The commercial formulation of TMC435 (G028) is an immediate release, oral, hard gelatin capsule containing 154.6 mg (b) (4) (as the Na-salt of the drug substance), which is equivalent to 150 mg of the (b) (4) drug substance.

(b) (4)

Table 2 Target Composition of the 150-mg Capsule

Component	Quality Reference ^a	Function	Quantity per Capsule (mg)
Simeprevir (b) (4)	Control of Critical Steps and Intermediates	Active	154.60
Sodium lauryl sulphate	Ph. Eur., NF	(b) (4)	(b) (4)
Magnesium stearate ^b	Ph. Eur., NF		
Colloidal anhydrous silica	Ph. Eur., NF		
Croscarmellose sodium	Ph. Eur., NF		
Lactose monohydrate	Ph. Eur., NF		
Nominal weight:			250.00
Hard gelatin capsule white body/white cap with black "TMC435 150" print	(b) (4)	Capsule	1 piece

^a Where multiple compendia are listed, the compendium that is applied, is specific to the applicable region of the submission.

Formulation used in nonclinical studies

In the nonclinical studies, TMC435 was generally used as (b) (4) the sodium (Na)-salt. Two formulations were used for oral gavage administration: TMC435 (b) (4)

The Na-salt of TMC435 was tested via the dietary route in mice and rats, in order to increase exposures and to avoid mortality caused by entry of the irritant and viscous formulation into the respiratory tract upon administration via oral gavage.

2.4 COMMENTS ON NOVEL EXCIPIENTS

None.

2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN

Five identified impurities in the drug substance at levels above the qualification threshold were:

(b) (4)

The impurities were qualified in the 6-month dog toxicity study as well as a 2-week oral rat toxicity study (1986_0027332). The drug substance impurities appear to be qualified at multiples of approximately (b) (4) the clinical dose.

The only impurity specified in the drug product is (b) (4) to not more than (b) (4). This impurity was qualified in the 6-month dog study at an approximate (b) (4) multiple over the clinical dose and was found not to be mutagenic (Study TOX9694).

Additionally, the sponsor identified potential genotoxic and known carcinogenic impurities in the drug substance synthesis process as summarized in the sponsor's table below.

Table 3 Summary of Potential Genotoxic and Known Carcinogenic Impurities in the Drug Substance Synthesis Process

(b) (4)

The starting material (b) (4) were all below the permissible daily exposure. (b) (4) were not tested and the sponsor notes that based on a scientific risk assessment, potential carryover to the final drug substance can be ruled out. This reviewer agrees with the sponsor that (b) (4) are unlikely to carry over to the drug product and/or pose a risk to patients.

The solvents used during the synthesis of the drug substance were present at levels below the defined ICH limits.

2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN

Proposed clinical population: treatment of chronic hepatitis C (CHC) genotype 1 infection, in combination with peginterferon alfa and ribavirin, in adults with compensated liver disease (including cirrhosis) who are treatment-naïve or who have failed previous interferon therapy (pegylated or non-pegylated) with or without ribavirin.

Proposed dose regimen: One capsule of 150 mg taken orally once daily with food for 12 weeks in combination with peginterferon alfa and ribavirin.

2.7 REGULATORY BACKGROUND

TMC435 was evaluated in clinical trials under IND 75391.

3 STUDIES SUBMITTED

3.1 STUDIES REVIEWED

Secondary Pharmacology

- NC225 *In vitro* interaction of 30 μ M TMC435350 (22.5 μ g/ml) with 50 receptors/channels was determined
- NC101 (b) (4) PharmaScreen
- NC 102 Effects of TMC435350 on the membrane K⁺ current (IKr) in HERG-transfected HEK293 cells compared to astemizole
- NC103 Electrophysiological effects of TMC435350 in isolated, Langendorff-perfused rabbit hearts
- NC104 Cardio-hemodynamic, cardio-electrophysiological and pulmonary effects of TMC435350 (0.16 – 5 mg/kg i.v.) in mechanically-ventilated, anesthetized dogs
- NC151 Effects of TMC435350 on the isolated, spontaneously beating right atrium of the guinea-pig.
- NC153 *In vitro* effects of TMC435350 on human platelet function using collagen, adenosine diphosphate and arachidonic acid as agonists for platelet aggregation.
- NC191 Effects of TMC435350 and of lidocaine on the membrane Na⁺ current in CHO cells stably transfected with hH1a cDNA
- NC192 Oral Cardiovascular and Respiratory Safety Study with TMC435350 in the Conscious Beagle Dog.
- NC196 RBC hemolysis Effect of TMC435350) on *in vitro* human red blood cell hemolysis.
- NC200 Single Dose Oral Safety Pharmacology Study of TMC435350 in the Rat: Modified Irwin's Test.
- NC229 Effects of the hepatitis C virus (HCV) protease inhibitor TMC435350 on gastric emptying in rats.

Pharmacokinetics

Absorption

- 1986_0036728 Pharmacokinetics of TMC435 in male beagle dogs after single IV administration of a PEG400 solution of TMC435 at 5, 10 and 20 mg eq./kg
- 1986_0036729 Pharmacokinetics of TMC435 in male Sprague Dawley rats after single IV administration of a PEG400 solution of TMC435 at 5, 10 and 20 mg eq/kg
- NC108 Pharmacokinetics, tissue distribution and absolute bioavailability in male SPF Sprague-Dawley rats after single oral administration of a VitE-TPGS/PEG-400 solution of TMC435350 at 40 mg/kg and after single intravenous administration of a 20% HP- β -CD solution of TMC435350 at 4 mg/kg.

- NC109 Pharmacokinetics and absolute and relative bioavailability in male beagle dogs after single intravenous administration of an aqueous 20% HP- β -CD solution of TMC435350 at 2 mg/kg and after single oral administration of TMC435350 as a solution in PEG400 with 2.5% Vit. E TPGS, at 5 mg/kg in fed and fasted conditions
- NC110, Evaluation of the Oral Bioavailability and Pharmacokinetics of TMC435350 in Male Cynomolgus Monkeys Following a Single Oral and Intravenous Bolus Administration
- NC113 Study on the transepithelial transport of TMC435350, the role of P-glycoprotein (P-gp) in the transepithelial transport of TMC435350 across Caco-2 monolayers and on the possible inhibition of human intestinal P-gp by TMC435350.
- NC208 Pharmacokinetics and relative bioavailability of TMC435350 in female New Zealand White rabbits after single oral administration of two different formulations of TMC435350 at 60 mg.
- NC209 Pharmacokinetics and relative bioavailability of TMC435350 in female New Zealand White rabbits after single oral administration of a 0.5% Methocel suspension of TMC435350 at 40 mg/kg, and after single oral, intravenous and subcutaneous administration of a nanosuspension of TMC435350 at 40, 4 and 40 mg/kg, respectively
- NC210 PK of TMC435350 After a Single Oral Administration as Different Formulations in Female Beagle Dogs
- NC211 PK and relative Bioavailability of TMC435350 in beagle dogs after single oral administration of different capsule and tablet formulations at 200 mg of TMC435350
- NC232 Pharmacokinetics and absolute bioavailability of TMC435350 in male syrian hamsters after single intravenous administration of a 20% HP- β -CD solution of TMC435350 at 8 mg/kg, and after single oral administration of a PEG400 solution of TMC435350 at 100 mg/kg.
- NC235 Pilot study on relative bioavailability and pharmacokinetics of TMC435350 following oral administration of TMC435350 (as free acid, as Na-salt and as HCl salt in pellets) by dietary admixture for 3 days to male mice.
- NC237 Pharmacokinetics of TMC435350, in male beagle dogs after single oral administration of several formulations at 200 mg/dog.
- NC277 Pharmacokinetics of TMC435, in male minipigs after single oral administration of several formulations at 3 mg/kg or at 100 mg/pig.
- NC162 Pharmacokinetics and relative bioavailability of TMC435350 in male beagle dogs after single oral administration of different formulations at 20mg/kg or capsules of 200 mg.
- NC163 Pharmacokinetics and relative bioavailability of TMC435350 in male beagle dogs after single oral administration of different formulations at 20mg/kg or capsules of 200 mg.

Distribution

- NC111 The protein binding of 3H-TMC435 in plasma collected in a phase 1, open-label trial to investigate the effect of severe renal impairment on the pharmacokinetics and safety of TMC435.

- NC166 Tissue distribution of ¹⁴C-TMC435350, as studied by whole-body autoradiography, in the pigmented male rat after single oral administration of ¹⁴C-TMC435350 at 120 mg/kg.
- NC202 The plasma protein binding of ³H-TMC435350 in animals and man
- NC213 Tissue distribution and placental transfer of ¹⁴C-TMC435350, as studied by whole-body auto radiography in the Sprague Dawley rat after single oral administration of ¹⁴C-TMC435350 at 120 mg/kg.
- NC249 Tissue distribution of ¹⁴C-TMC435350, as studied by whole-body autoradiography in the pigmented male mice after single oral administration of ¹⁴C-TMC435350 at 150 mg/kg.
- NC213 Tissue Distribution and Placental Transfer of ¹⁴C-TMC435350, as Studied by Whole-Body Autoradiography in the Sprague-Dawley Rat after Single Oral Administration of ¹⁴C-TMC435350 at 120 mg/kg.

Metabolism

- 1986_0049051 *In Vitro* study on the Inhibition of Cathespin A by TMC435
- NC112 The *in vivo* metabolism of TMC435350 in male SPF Sprague-Dawley rat and male Beagle dog.
- NC115 Estimation of the effect of TMC435350 on the metabolism of co-administered drugs by the determination of the K_m and V_{max} for TMC435350 in human liver microsomes
- NC114 The *in vitro* metabolism of TMC435350 in hepatocytes and liver subcellular fractions of male and female mouse, male and female rat, female rabbit, male dog, male cynomolgus monkey and man
- NC121 An *in vitro* study to assess the potential of TMC435350 to induce CYP enzyme activities in cryopreserved human hepatocytes.
- NC168 The *in vitro* metabolism of ¹⁴C-TMC435350 in liver microsomes and hepatocytes of male mouse, male rat, female rabbit, male dog, male monkey and human.
- NC169 The metabolism and excretion of ¹⁴C-TMC435350 in male and female Sprague-Dawley rats after single oral administration of ¹⁴C-TMC435350 at 120 mg/kg.
- NC212 The absorption, metabolism and excretion of ¹⁴C-TMC435350 in the male Beagle dog after a single oral dose of ¹⁴C-TMC435350 at 30 mg/kg.
- NC215 Study on the possible induction and/or inhibition of hepatic drug metabolising enzymes by TMC435 in male and female SPF Albino Swiss mice
- NC220 Study on the possible induction and/or inhibition of hepatic drug metabolising enzymes by TMC435 in male and female beagle dogs
- NC231 LC-MS identification of the metabolites in mouse plasma after single and multiple oral administration of TMC435350.
- NC273 An *in-vitro* study on the inhibition of UGT1A1 mediated bilirubin glucuronidation by TMC435 and Ribavirin in human liver microsomes
- NC116 An *in vitro* study to determine the kinetics of TMC435350 metabolism in human liver microsomes, and to identify the microsomal cytochrome P-450 iso-enzymes mediating TMC435350 metabolism (reaction phenotyping).

- NC117 An *in-vitro* study on the direct inhibition of the metabolism of the cytochrome P-450 probe substrates by TMC435350
- NC107 A study of the effects of TMC435350 on some hepatic enzyme activities after oral administration for one month at doses of 0, 50, 150 and 500 mg/kg/day to male and female Sprague-Dawley rats.

Excretion

- NC165 The metabolic stability, metabolism and excretion of TMC435350 in the male SPF Sprague-Dawley rat after a single oral dose of ¹⁴C-TMC435350 at 120 mg/kg.microsomes
- NC194 The biliary excretion of TMC435350 in male SPF Sprague-Dawley rats after a single oral administration at 40 mg/kg

Drug interaction studies

- NC155 Pharmacokinetics of TMC435350 and boosting effect of Ritonavir in fed male beagle dogs after single oral administration of TMC435350 at approximately 2 and 5 mg/kg.
- NC197 An *in-vitro* study on the inhibitory effect of TMC435350 on the metabolism of potential co-medications

PK/ADME Other

- 1986_0029246 Pharmacokinetics of TMC435 in the male wild-type FVB mice and male Oatp1a/1b ^{-/-} mice
- 1986_0029247 The uptake of TMC435 in HEK293 cell lines overexpressing the transporters OATP1B1, OATP1B3, OATP2B1 or OATP1B1*15
- NC239 ABCB1, Abcg2 and ABCC2 mediated transport of TMC435 in LLC-PK1 (ABCB1) and MDCKII (Abcg2, ABCC2) cell lines
- NC241 *In vitro* study on the uptake transport of TMC435 in rat and human hepatocytes, the effect of ritonavir, rifampicin and cyclosporine A on TMC435 uptake and the effect of TMC435 on hepatic uptake of taurocholate and estradiol 17 β -D-glucuronide
- NC242 Assessing transport inhibition of TMC435 on ABCC2 (MRP2) and ABCB11 (BSEP) using inside-out vesicles.
- NC275 Assessing transport inhibition of TMC435 on ABCC2 (MRP2) and ABCB11 (BSEP) using inside-out vesicles.
- NC282 The effect of TMC435 on transport of 17 β -estradiol-glucuronide and taurocholate mediated by OATP1B1 (SLCO1B1) and NTCP (SLC10A1) in CHO cell lines overexpressing these transporters.

Genetic Toxicity

- NC148 *In Vitro* Bacterial Reverse Mutation Test with TMC435350 in *Salmonella typhimurium*
- NC173 *In Vitro* Bacterial Reverse Mutation Test with TMC435350 in *Salmonella typhimurium*
- NC130 *In Vitro* Mammalian Forward Mutation Test with L5178Y Mouse Lymphoma Cells (TK-locus) using the Microtiter® Fluctuation Technique with TMC435350

- NC172 *In vivo* micronucleus test with TMC435350 on bone marrow cells of mice.

General Toxicology:**Mouse**

- NC205 14 day repeat dose oral in mice
- NC251 TMC435350: Preliminary Toxicity Study by Dietary Administration to CD-1 Mice for 17 Days – NOT REVIEWED
- NC230 2-Week Repeated Dose Dietary Toxicity Study of TMC435350 in the Mouse.
- NC253 Supplementary Toxicity Study by Dietary Administration to CD-1 Mice for 13 weeks (3 g/kg/day)

Rat

- NC140 Single Dose Oral Toxicity Study followed by a 6-Day Repeated Dose Oral Toxicity Study in the Rat (tolerability study)
- NC144 2-Week Repeated Dose Oral Toxicity Study of TMC435350 in the Rat
- NC177 1-mos Repeated Dose Oral Toxicity Study of TMC435350 in the Rat with a 1-mos Recovery Period.
- NC236 2-Week Repeated Dose Dietary Toxicity Study of TMC435350 in the Rat.
- NC244 Toxicity Study by Dietary Administration to CD Rats for 13 Weeks
- NC179 6-mos Repeated Dose Oral Toxicity Study of TMC435350 in Rat w/ 3 mos interim kill

Dog

- NC145 Single dose Escalation Oral Toxicity Study of TMC435350 in the Beagle Dog Followed by a 5-day Repeated Dose Oral Toxicity Study (Tolerability Study)
- NC161 2-wk oral (gavage) toxicity study in beagle dogs
- NC175 1-mos Repeated Dose Oral Toxicity Study of TMC435350 in Beagle Dog w/ 1-mos Recovery Period.
- NC207 TMC435350: Toxicity Study by Oral Gavage Administration to Beagle Dogs for 39 Weeks Followed by a 13 Week Recovery Period
- NC226 TMC435350: Toxicity Study by Oral Gavage Administration to Beagle Dogs for 6months

Monkey

- NC181 TK and escalating IV and oral (by gavage) dose range finding study followed by an oral 14- or 28- day fixed dose phase in the male rhesus monkey

Reproductive Toxicology

- NC190 - Fertility study of oral TMC435350 in the male and female rat.
- NC185 - Pilot oral developmental toxicity study of TMC435350 in the rat.
- NC187 - Pilot oral developmental toxicity study of TMC435350 in the mouse.
- NC188 Oral Developmental Toxicity Study of TMC435350 in the Rat.
- NC189 Embryo–Fetal Toxicity Study by Oral Gavage Administration to CD-1 Mice.
- NC224 TMC435350: Pre- and Post-Natal Development Study in the CD Rat by Gavage Administration
- NC227 TMC435350: Preliminary Pre- and Post- Natal Development Study by Oral Gavage Administration to CD Rats

Other Studies

- NC114 The cytotoxicity of TMC435350 in rat, dog, cynomolgus monkey and human hepatocytes.
- NC234 *In Vitro* Bovine Corneal Opacity-Permeability Irritation Test

Local Tolerance

- NC131 *In Vitro* Bovine Corneal Opacity-Permeability Eye Irritation Test
- NC174 Local lymph node assay in mice with TMC435350
- NC203 Primary skin irritation study in rabbits
- NC216 Cytotoxicity assay in vitro with balb/c 3T3 Cells – neutral red test with TMC435350
- NC255 Cytotoxicity assay in vitro with balb/c 3T3 Cells – neutral red test with TMC435350 in presence of a protein supplement during simultaneous irradiation with artificial sunlight

Impurity Studies:

Full reviews were not conducted for these studies.

- 1986-16572 *In Vitro* Bacterial Reverse Mutation Test with TMC435 spiked with 5% (b) (4) in *Salmonella typhimurium*
- 1986-16574 TMC435350 + 5% (b) (4): *In Vitro* Mutation Test using Mouse Lymphoma L5178Y Cells
- 1986-0022221 *In Vitro* Bacterial Reverse Mutation Test with TMC435350 spiked with 5% (b) (4) and 5% (b) (4) in *Salmonella typhimurium*
- 1986-0022222 90% TMC435350 + 5% (b) (4) & 5% (b) (4) *In Vitro* Mutation Test using Mouse Lymphoma L5178Y Cells
- 1986_0022223 *In Vitro* Bacterial Reverse Mutation Test with TMC435350 spiked with 5% (b) (4) and 5% (b) (4) in *Salmonella typhimurium*
- 1986_0022224 90% TMC435350 + 5% (b) (4) & 5% (b) (4): *In Vitro* Mutation Test using Mouse Lymphoma L5178Y Cells
- 1986_0022225 *In Vitro* Bacterial Reverse Mutation Test with TMC435350 spiked with 5% (b) (4) and 5% (b) (4) in *Salmonella typhimurium*
- 1986_0022226 90% TMC435350 + 5% (b) (4) & 5% (b) (4) *In Vitro* Mutation Test using Mouse Lymphoma L5178Y Cells
- 1986_0027332 2-week Repeated Dose Oral Toxicity Study of TMC435350 in the Rat.

3.2 STUDIES NOT REVIEWED

The following studies were reviewed briefly but not included in this review because they were not pivotal safety studies.

- NC257 2-week Repeated Dose Oral Toxicity Study of TMC435350 in the Rat.
- NC259 Seven days repeated dose Toxicity Study of TMC435350 in the Rat.
- NC252 TMC435350: Pilot Toxicity Study by Dietary Administration to CD Rats for 17 Days
- NC128 Determination and assessment of the potential of TMC435350 to induce damage to the chromosomes or the mitotic apparatus in peripheral blood

reticulocytes (micronucleus test) and determination of systemic exposure to TMC435350 when administered once orally by gastric intubation to male CD-1 mice. The toxicities identified in the 3-month mouse studies (NC233 and NC206) were consistent with those in NC253 which achieved higher exposure, therefore NC233 and NC206 were not reviewed in detail:

- NC233 Toxicity Study by Dietary Administration to CD-1 Mice for 13 Weeks (0.5, 2 or 5 g eq./kg/day)
- NC206 3-Month Repeated Dose Oral Toxicity Study of TMC435350 in the Mouse
The analytical method validation studies were not reviewed as no issues were identified with the analytical data.
- Analytical Method Validation Studies – NC125, NC127, NC157, NC193

3.3 PREVIOUS REVIEWS REFERENCED

Reviews by Christopher Ellis, PhD for IND 75391.

4 PHARMACOLOGY

TMC435 is an inhibitor of the HCV NS3/4A protease, with a median inhibition constant value (K_i) of 0.5 and 1.4 nM against the HCV genotype 1a and genotype 1b protease *in vitro*, respectively. The *in vitro* anti-HCV median 50% effective concentration (EC_{50}) and EC_{90} of TMC435 is 9.4 and 19 nM, in the genotype 1b Huh7-Luc cells with luciferase readout, respectively. The EC_{50} values ranged from 3.7 to 25 nM for genotype 1b and were 23 and 28 nM for genotype 1a replicon containing cells using RT-PCR read out.

The primary pharmacology is reviewed in detail in the Clinical Virology review. The mean C_{max} from the Phase 2b clinical trials was used for comparison to the concentrations/doses of TMC435 used in the *in vitro* pharmacology studies. Values for C_{max} were 4394 ng/mL for trial C205 [n=23] and 3953 ng/mL for trial C206 [n=26], which gives a mean C_{max} of 4160 ng/mL).

4.1 PRIMARY PHARMACOLOGY

See Clinical Virology review by Damon Deming, PhD.

4.2 SECONDARY PHARMACOLOGY

In vitro binding to various receptors was evaluated in two different screening assays (study reports NC101 and NC225).

The *in vitro* interaction of 30 μ M TMC435350 (22.5 μ g/ml) with 50 receptors/channels was determined (NC225). TMC435 inhibited receptors that are distributed in the CNS, the cardiovascular system, and the gastrointestinal tract. Of the receptors/channels that demonstrated a strong interaction, only 68%, 89%, and 96% inhibition of the A_1 adenosine receptor, A_3 adenosine receptor, and angiotensin II receptor (AT_1), respectively, could be relevant physiologically based on TMC435350 biodistribution. Potential cardiovascular effects of receptor inhibition include reduced coronary blood flow (vasoconstriction), reduced blood pressure and diuresis, the latter two due to vasodilation (reduced vascular resistance) and reduced secretion of vasopressin and aldosterone. Inhibition of CNS receptors were identified; however, based on the *in vivo*

radiolabeled distribution studies very little TMC435 distributes to the CNS. Finally, TMC435 was found to inhibit cholecystokinin (CCK) by 60% which is proposed by the sponsor to be associated with delayed gastric emptying in rats and the pancreas findings (decreased acinar cell zymogen/basophilia, acinar cell vacuolation, increased lipase and amylase levels, inflammation and apoptosis) in rats, mice and dogs. CCK is involved in the digestion of fat and protein. It is synthesized by cells in the duodenum and secreted in the blood and causes the release of digestive enzymes and bile from the pancreas and gall bladder.

4.3 SAFETY PHARMACOLOGY

TMC435 was evaluated in central nervous system (CNS), respiratory, and cardiovascular safety pharmacology studies.

In the rat CNS safety pharmacology study (NC200), neurological effects included diminished alertness, narrowing of the palpebral fissure, and in one rat at the high dose of 500 mg/kg, clonic jaw muscle contraction. Following a single dose in the mouse (NC172), a decrease in general activity, narrowing of the palpebral fissure, and piloerection were seen at 1000 and 2000 mg/kg.

Cardiovascular safety pharmacology studies included in vitro studies as well as in vivo studies in the dog. The in vitro studies showed a potential inhibitory effect on membrane Na⁺ channel current (hH1a, human heart sodium channel) at concentrations as low as 70 ng/ml. In the isolated Langendorff-perfused rabbit heart assay, proarrhythmic effects appeared to correlate with high levels of drug accumulation in the heart (median 597 µg/g). The in vivo dog studies showed increased vascular resistance, decreased heart rate, decreased cardiac output as well as an increase in the RR-interval. Although electrophysiology studies resulted in no clear cardiotoxicity signal, in the 2-week repeat dose toxicology study, cardiac necrosis was observed indicated the heart is a potential target organ of TMC435 toxicity.

Respiratory effects were not observed in dogs or rats.

A delay in gastric emptying was observed in rats 1 hr following oral doses of TMC435 from 160 mg/kg-640 mg/kg.

The safety pharmacology studies are described in more detail in the review by Christopher Ellis, PhD below:

Neurological effects: Studies in Sprague-Dawley rats (♂) (n=5) using a Modified Irwin's test, given single oral doses of 0, 50(2.3), 150(2.8), 500(3.5) mg/kg (C_{max}) followed by a 7d observation period, produced diminished alertness at all doses between 2 and 6 hr post-dose and a dose-dependent narrowing of the palpebral fissure beginning at 150 mg/kg [3 on 0-4 scale (2-3/5 at MD & 4/5 at HD), HC=88% at 4, 12% at 3] in the absence of signs of general toxicity. One rat, given 500 mg/kg, displayed clonic jaw muscle contraction at 1 hr post-dose (NC200). Drug-related effects on the CNS are not expected, given that TMC435350 is not distributed to the rat brain (see PK section).

Additionally, single oral dose studies in mice showed a small transient decrease in general activity, narrowing of the palpebral fissure and piloerection at 1000 mg/kg [C_{max} =12.8(♂)-29.5(♀) µg/ml] and 2000 mg/kg [C_{max} =35.7(♂)-60.7(♀) µg/ml] (NC172).

Cardiovascular effects:

In vitro studies:

- No significant effect on membrane K^+ current with concentrations up to 3 µM detected in HEK293 cells transfected with hERG, although only 13% of 3 µM was recovered in the bath solution (NC102).
- 10 to 60% reductions in membrane Na^+ current were observed with TMC435350 concentrations of 0.07(0.1) 0.22(0.3), 0.75(1), 2.25(3) and 7.5(10) µg/ml(µM) in CHO cells transfected with human hH1a (NC191). Conclusion: Although TMC435350 has a higher affinity for the hH1a channel than lidocaine, the dose response curve is flat (nonspecific partial inhibitor). Additionally, this inhibition is probably not significant *in vivo*, because increased QRS width and/or P-R prolongation was not observed in anesthetized (NC104) or conscious (NC192) dog studies.
- The guinea pig spontaneously beating atrium assay gave unreliable results due to low drug exposure (3% of 10 µM) (NC151).
- The isolated Langendorff-perfused rabbit (♀) heart assay was performed following two protocols. For the 30 min perfusion protocol, observations included 16, 35 and 50%↑ in coronary flow for 1, 3, and 10 µM concentrations, respectively, without effects on triangulation, instability, IVC, or arrhythmias (EADs, VT, VF, & TdPs). A shortened APD_{60} (19%) was also observed at 10 µM. For the 2nd protocol, hearts were perfused for 60 (3 µM) min followed by 90 (10 µM) min. A shortened APD_{60} (19%) and a 23%↑ in coronary flow was observed at 3 µM. Multiple problems arose following the 90 min perfusion with 10 µM TMC435350 including various arrhythmias (VF in 4/6); however, it was determined that this was due to massive accumulation of drug in heart tissues during the perfusion period (median=597 µg/g) (NC103). Conclusion: Proarrhythmic effects are due to drug accumulation and are not relevant *in vivo* due, in part, to high plasma protein binding of TMC435350 (see PK section).

In vivo studies:

- Studies in anesthetized dogs (n=4), given escalating IV doses of 0.16 to 5 mg/kg (C_{max} =67.2 µg/ml), showed a tendency for mild cardio-hemodynamic or cardio-electrophysiological effects over a 30 min interval (↑VR & ↓HR/CO) [only statistically significant change: 21%↑ RR-interval in 1.25 mg/kg (C_{max} =12.2 µg/ml) group] (NC104).
- No adverse effects were observed in cardiovascular (HR, BP and pressure rate) or EKG (RR-,PQ- and QT-intervals, QRS complex) parameters over an 8 hr post-dose period in conscious dogs (n=4) given escalating oral doses of 10, 40 or 160 mg/kg [6.2(27.8), 51.3(228), and 90.8(301) µg/ml(µg.h/ml), for C_{max} (AUC)] (NC192). NOAEL=160 mg/kg.

- No effects on EKG parameters were observed in dogs in 2 wk (NC161) or 1 mos (NC175) repeat-dose oral toxicity studies given 0, 10, 40, or (120)160 mg/kg or 0, 10, 30, 90 mg/kg, respectively. However, cardiac necrosis was observed in the 2 week repeat dose dog study.

Pulmonary effects: Studies in anesthetized (NC104) and conscious (NC192) dogs revealed no adverse effects on pulmonary parameters including respiratory rate and arterial blood oxygen saturation rate. Additionally, no effects were observed in rats given single oral doses (NC172).

Gastrointestinal effects: TMC435350 inhibited gastric emptying (gastric content was increased ~2.5-fold 1 hr after test meal) in a non-dose dependent manner in Wistar rats given oral doses of 0, 160, 320, 640 mg/kg as determined using the Choco test (NC104).

Other: *In vitro* platelet aggregation (NC153) and RBC hemolysis (NC196) assays using human blood were also performed. No significant effects of TMC435350 on aggregation or hemolysis were observed in either assay up to 30 μ M and 300 μ M, respectively.

5 PHARMACOKINETICS/ADME/TOXICOKINETICS

Pharmacokinetic studies of TMC435 were conducted by the oral, intravenous and subcutaneous (rabbit only) routes in various species that included mice, rats, hamsters, rabbits, minipigs, dogs and monkeys. TMC435

(b) (4)

(b) (4)

Overall, the bioavailability of TMC435 was moderate to high across nonclinical species: rat - 44%, dog - 72%, monkey - 25%, hamster - 40%, minipig - 19-63%, with the exception of the rabbit (2.5%). T_{max} generally occurred between 1-5 h, absorption and/or elimination appeared to saturate at higher doses, clearance (Cl) was low in the monkey and dog (0.2-0.4 L/kg), moderate in the rat and hamster (2.3 L/kg), and high in the rabbit (7.2 L/kg) which likely explains the poor bioavailability of TMC435 in the rabbit. TMC435 is primarily metabolized by CYP3A4 with unchanged drug as the predominant moiety in plasma. No major (10% or greater) metabolites were identified in human or nonclinical species. Although not a major metabolite, M21 was the highest human circulating metabolite at 8% of the mean plasma AUC, after a single oral dose of 200 mg TMC435 in healthy human volunteers. M21 was detected in both rat and human plasma. TMC435 showed inhibition of CYP2A6, CYP2C8, CYP2D6, CYP2C19 and CYP3A in human liver microsomes. Although induction of CYP1A2 or CYP3A4 was not seen in human hepatocytes, induction of various CYPs was observed in ex vivo liver

microsomes from studies in the mouse (CYP4A, CYP3A, CYP2E, and thyroxine UDPGT), rat (CYP2B1, CYP2E1, CYP3A4) and dog (CYP4A and CYP1A).

TMC435 is highly bound to albumin (>99.9% across nonclinical species and >97.2-99.7% in human), predominantly distributes to the liver and gastrointestinal tract and is primarily excreted in the feces across species. TMC435 is a substrate of the uptake transporters OATP1B1, OATP1B3, OATP2B1, and efflux transporters P-gp/MDR1, MRP2, and BCrp1. TMC435 is an inhibitor of OATP1B1, NTCP, P-gp/MDR1, BSEP, and MRP2.

5.1 PK/ADME

Absorption

TMC435 has low permeability in the human intestine and is an inhibitor of P-gp (MDR1 or ABCB1, IC50-value of 85.9 μ M or 64.4 μ g/ml (Study NC113). TMC435350 is highly bound to serum albumin (Study NC202) across species: healthy male subjects (>97.22->99.69%), cynomolgus monkeys (>99.97%), beagle dogs (>99.99%), New Zealand white rabbits (>99.99%), Sprague Dawley rats (>99.99%), and Swiss-CD1 mice (>99.99%) (Study NC111).

In vivo

The single-dose PK parameters are summarized below; the mouse and rabbit PK data are from repeat-dose studies. The repeat dose TK data are reviewed in Section 6 General Toxicology.

Mouse: The single-dose oral PK of TMC435 in the mouse was evaluated after the first dose of a 2-week repeat dose oral gavage toxicity study and in a 3-month repeat dose oral gavage toxicity study at doses from 150-2000 mg/kg/day. T_{max} ranged from 3-8 h. C_{max} did not increase with dose and appeared to be similar (<2-fold difference) across the dose range for the 2-week and 3-month studies although the selected doses have a 13-fold separation between the low and high dose. With increasing dose, AUC increased less than dose-proportionally.

Rat: The PK of TMC435 in a PEG400 solution in the rat following a single IV dose at 5, 10 and 20 mg/kg resulted in approximately dose proportional increases in C_{max} and AUC between the low dose and the mid-dose, and greater-than-dose-proportional increases in AUC (~6X) and C_{max} (~8X) at the high dose. The $T_{1/2}$ was ~5.4 hours, the total plasma Cl was 1.1-1.6 L/h/kg, with a decrease in the steady state volume of distribution with increasing dose from 4.09 L/kg at the low dose to 2.77 L/kg at the high dose. Following a single oral dose of a VitE-TPGS/PEG400 solution at 40 mg/kg, the T_{max} was 2 hours with a $T_{1/2}$ of 2.6 hours, an AUC of 7750 ng*hr/ml and oral bioavailability (F) estimated to be 44%.

Dog: Following a single IV dose of TMC435350 at 5, 10 and 20 mg/kg, (Study No. 1986_0036728), greater-than-dose-proportional increases in AUC (a 10.7-fold difference between the low and high dose), dose-proportional increases in C_{max} , an increase in the terminal half-life with increasing dose levels (from 2.78 h at the low dose

to 4.74 h at the high dose), a decrease in clearance (Cl) at higher dose levels (from 0.191 L/h/kg at the low dose to 0.073 L/h/kg at the high dose), as well as a decrease in the volume of distribution with increasing dose (0.451 L/kg at the low dose to 0.247 L/kg at the high dose) were observed. The decrease in Cl with increasing dose may indicate a saturating effect on the clearance of TMC435. Plasma levels were below the LLOQ by 31 or 48 hours post-dose. The average bioavailability of TMC324 in fed dogs was 71.6%; fed dogs had slightly higher relative bioavailability (124%) compared with fasted dogs (Study NC109).

Monkey: Following a single IV dose, TMC435 exhibited low Cl in the monkey (10% of hepatic blood flow) with a $T_{1/2}$ of 6.43 hours, and limited distribution outside of plasma (V_{dss} 456 mL/kg, less than total body water). After a single oral dose of 20 mg/kg, absorption was moderate with a T_{max} of 3.33 hours (fed) - 3.67 hours (fasted), and a $T_{1/2}$ of 4.5 hours (fasted) - 6.07 hours (fed). Under fed conditions C_{max} (48% decrease) and AUC (26% decrease) were lower. The bioavailability was slightly greater under fasted (25%) vs. fed (19%) conditions (Study No. NC110).

PK studies in the rabbit, minipig and hamster were conducted for potential evaluation in toxicity studies. Ultimately, these species were not utilized in the toxicity studies so the results from these studies are only briefly summarized.

Rabbit: Oral dosing of TMC435 in a 0.5% methocel suspension or as a Na salt in a capsule showed low concentrations with only a limited number of samples above the LLOQ (Study NC208). Beyond 5-7 hours after dosing, plasma levels were not detected. Relative bioavailability (F) was 2.5%. Following IV dosing, clearance was high (7.2 L/h/kg) compared with hepatic (4.25 L/h/kg) and renal (1.92 L/h/kg) blood flow, and a high volume of distribution (40.8 L/kg) was observed indicating extensive tissue distribution. SC dosing resulted in measurable plasma levels for up to 14 days with an estimated complete release from the SC injection site of ~ 5 weeks and estimated F of 60%. **It is unclear why the SC dose route was not further explored for an embryofetal study in rabbit.**

Hamster: Following IV dosing, the V_d (5.9 L/kg) indicated extensive tissue distribution. Plasma Cl (2.26 L/h/kg) approximated hepatic (2.6 L/h/kg) and renal (2.48 L/h/kg) blood flow. Following a single oral dose at 100 mg/kg in a PEG400 solution, the T_{max} was 2-4 h, the $T_{1/2\ 24-31h}$ was 9.9h, with an absolute oral F of 39.7%. Exposure in the liver between 7-31 h was higher than plasma levels (Study NC232).

Minipig: The oral bioavailability of TMC435 in a capsule, tablet and (b) (4) Na-salt form ranged from 19-63%, with AUC values from ~120-400 ng*h/ml and C_{max} ranging from 36.5-58.5 ng/ml. The sponsor concluded that the minipig would not be good non-rodent model because the dose normalized exposure was much lower compared with the dog (Study NC277).

Decreased exposure with repeat doses of TMC435 in nonclinical species:

Although the repeat dose TK data are discussed in detail in the General Toxicology Section, it is important to note in the PK/ADME Section that decreased exposure with TMC435 was observed across multiple species including the mouse, rat, and dog following repeat doses of TMC435. At this time the mechanism behind the decreased exposure following repeat doses is not known but could be related to TMC435 metabolism autoinduction, clearance induction, delayed gastric emptying, or uptake inhibition. Decreased exposure following repeat doses was seen in the mouse (2-week dietary feed, 3-month dietary feed study, 3 month oral gavage), as well as the rat (1-month oral gavage and 6-month oral gavage) and the dog (1-month oral gavage, 6-month oral gavage, 9-month oral gavage) toxicology studies.

After observing decreased exposures of TMC435 in a 2-week repeat dose oral mouse toxicity study (TOX8459), the sponsor evaluated plasma samples from the 2-week mouse study to determine whether increased metabolism could account for the decreased exposure. Neither an increase nor a decrease in metabolite levels was observed (Study NC231). Liver samples from in vivo studies in the mouse, rat and dog showed induction of some CYP450 enzymes. Although induction of CYP1A2 or CYP3A4 was not seen in human hepatocytes, induction of various CYPs was observed in ex vivo liver microsomes from studies in the mouse (CYP4A, CYP3A, CYP2E, and thyroxine UDPGT), rat (CYP2B1, CYP2E1, CYP3A4) and dog (CYP4A and CYP1A). However, it is not known if CYP450 induction is a main or contributing mechanism to the decrease in exposure following repeat-doses.

Another possible mechanism is that repeat doses impact the absorption of TMC435. TMC435 has been shown to delay gastric emptying in a safety pharmacology study. The sponsor hypothesized that TMC435 could precipitate in the GI tract over time and although the nonclinical studies did show abnormal GI contents as a necropsy finding, more drug would need to be cleared by precipitation vs. absorption into the plasma to result in a decrease in exposure.

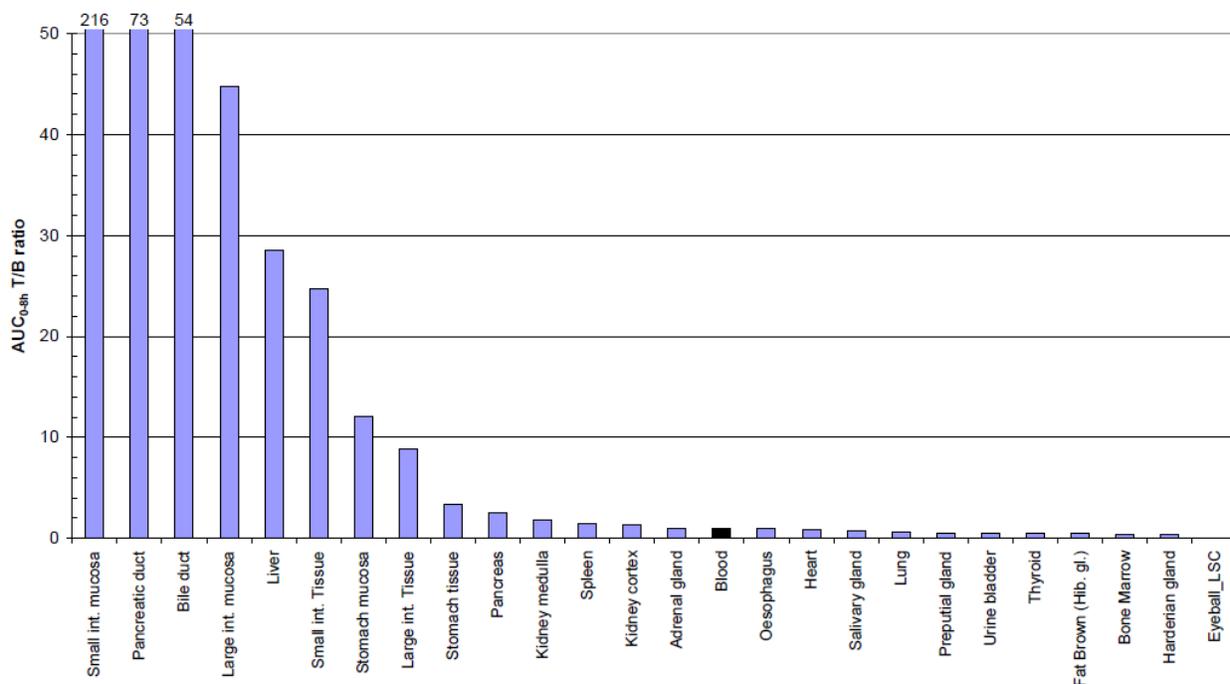
In conclusion, the mechanism of the decreased exposure following multiple doses is unknown in the nonclinical studies. This effect was not observed in clinical trials. Although decreased exposure with repeat dosing was observed, the toxicology studies appear to be valid since TMC435-related toxicity was seen in the pivotal studies.

Distribution

Radiolabeled tissue distribution studies of TMC435 were performed in the mouse, male rat, and pregnant female rat. Tissue distribution (Study NC166) after a single oral dose of ¹⁴C-TMC435350 at 120 mg/kg in the male pigmented rat using whole-body autoradiography showed drug distribution mainly to the liver, GI, pancreatic duct and bile duct. In non-pigmented tissues the highest levels of TMC435 (>25-fold the AUC in blood) were measured in GI tissues, liver, bile duct, and pancreatic duct. Intermediate levels (2-12-fold the AUC in blood) were measured in the stomach, large intestinal wall, pancreas, and renal medulla. Levels similar to blood were seen in the spleen, renal cortex, glandular tissues (adrenal, salivary, preputial and thyroid), oesophagus, heart, lung, and urinary bladder. Pigmented tissues (skin, uveal tract, eyeball, brain meninges)

showed very low to undetectable levels compared with blood. The data are summarized in the sponsor's figure below (Figure 1).

Figure 1 Tissue to blood (RLG) AUC0-8h-ratios of total radioactivity after single oral administration of ¹⁴C-TMC435350 at 120 mg/kg in the rat.



The tissue distribution of TMC435 in the pregnant rat (Study NC213) was previously reviewed by Dr. Chris Ellis. In non-procreative tissues, the distribution of TMC435 was similar to the male rat with distribution to the small intestine and the liver. In the procreative tissues, the highest tissue to blood AUC ratio was seen in the uterine epithelium. In the placental-fetal tissues and the mammary gland the AUC-values were comparable or lower than blood. In the fetal liver and the whole fetus total radioactivity was below the lower limit of quantitation.

The tissue distribution of TMC435 in the mouse overall was very similar to the rat with the highest distribution observed in the liver, GI and pancreas (Study No. NC249).

METABOLISM

In Vitro Metabolism

Enzymes of the CYP3A family, CYP3A4, CYP3A5 and CYP3A7 play a major role in the metabolism of TMC435. CYP2C8, CYP2C19, CYP2A6, CYP2B6 and CYP2E1 may also play a role but the in vitro results were not always consistent. TMC435 inhibited human CYP2D6, CYP2C8, CYP2A6, CYP2C19 and CYP3A4 with IC₅₀ values ranging from 32.2 µg/ml to 116 µg/ml (Study NC117). TMC435 did not induce CYP1A2 or CYP3A4 in vitro at 10 µM. TMC435 did appear to inhibit CYP3A4 levels and activity at 2.5 µM and 10 µM (Study NC121). TMC435 did not inhibit cathepsin A at clinically relevant levels (IC₅₀>50 µM, Study No. 1986_0049051).

The in vitro metabolism of radiolabeled ¹⁴C-TMC435350 was evaluated in liver microsomes and hepatocytes in the male mouse, male rat, female rabbit, male dog, male monkey and human (Study NC168). All human metabolites were seen in vitro and or in vivo in the rat and/or the dog. The M5 human metabolite was measured in human feces (Study C103), seen in vitro in the rat but not in vivo in nonclinical species. Although this metabolite was not measured in vivo in nonclinical species, it does not appear in circulation in humans. Therefore, potential safety concerns related to the M5 metabolite are unlikely. The major Phase 1 pathways of metabolism of TMC435 were O-demethylation and oxidation. The major Phase 2 pathway was glucuronidation of the oxidized metabolites.

The inhibition of UGT1A1-mediated bilirubin glucuronidation by TMC435 in pooled human liver microsomes (Study NC273) best fit an uncompetitive inhibition model with an apparent inhibition constant (K_i) of 119 μM +/-14μM; a concentration higher than the anticipated clinical C_{max} of 4.2 μg/ml. The in vitro study also determined that ribavirin was unlikely to interact with the glucuronidation of bilirubin.

In Vivo Metabolism

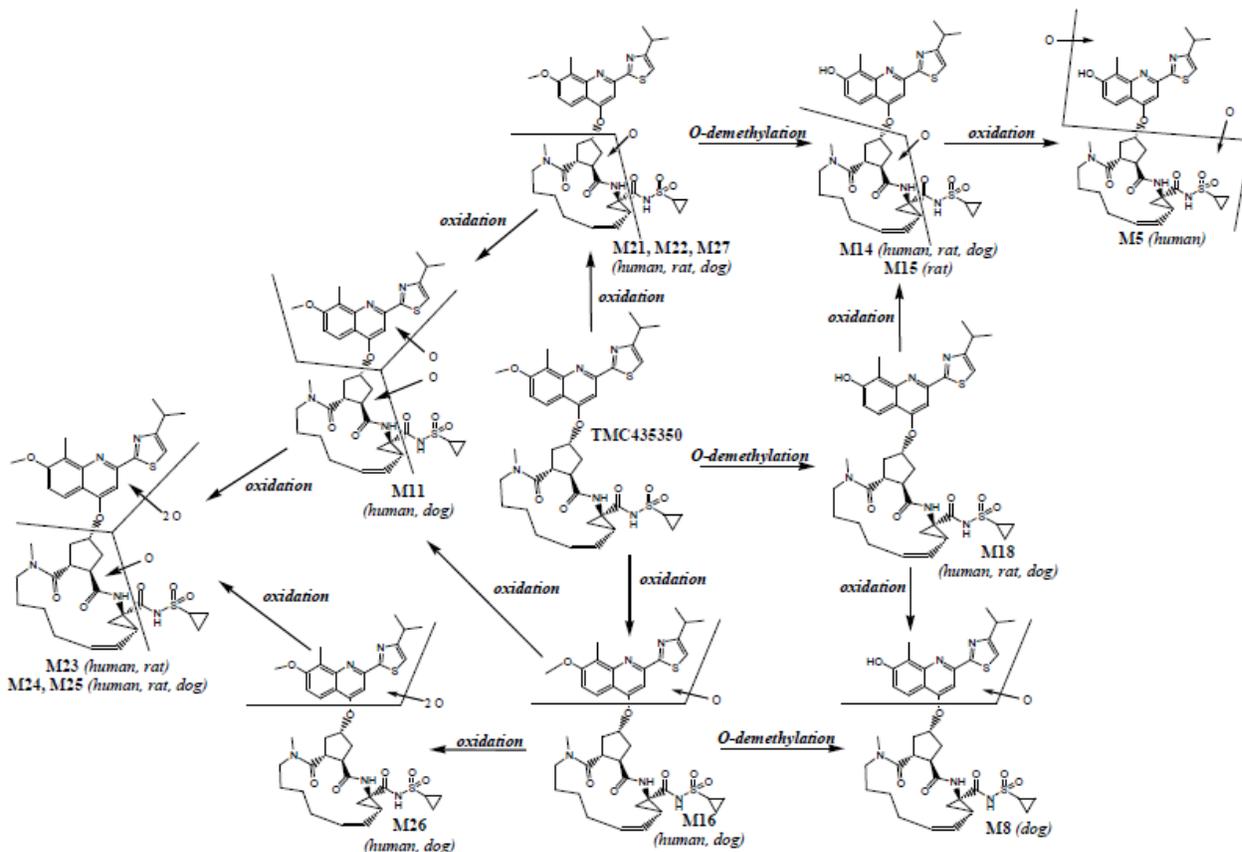
The proposed in vivo metabolism of TMC435 in rat, dog and human is summarized in Figure 2 below.

Mice: In SPF Albino Swiss Mice after oral doses of TMC 435 at 150, 500 and 2000 mg/kg/day for 3 months (Study NC215/FK6914), CYP4A was statistically significantly induced in males (1.4-1.7-fold), CYP2B was statistically significantly inhibited in females (up to 60%). Although not statistically significant, TMC435 appeared to inhibit CYP1A in female mice (up to 30%), induce CYP3A in male mice (up to 40%), induce CYP2E in male mice (up to 40%), and induce thyroxine UDPGT in female mice (up to 40%). There were no significant changes in total microsomal protein levels and total CYP450 levels.

Rats: The in vivo hepatic enzyme induction potential of TMC435 was evaluated in Sprague-Dawley rats after oral administration of TMC435 for one month at doses of 0, 50, 150 and 500 mg/kg/day (Study NC107). TMC435 in the rat was not a peroxisome proliferator, did not impact UDP-glucuronosyltransferase and was a weak inducer of CYP3A4 (2.2X) in female rats as well as CYP2B1 (2X) and CYP2E1 in male rats. Finally a decrease in CYP4A (80%) levels was seen in female rats at 50 mg/kg/day but not at higher doses.

Dogs: The in vivo hepatic enzyme induction potential of TMC435 in dogs was evaluated using liver samples (from 2 animals/sex/group) retained (stored frozen) from the 6-month dog oral repeat dose toxicity study (NC226) in Study NC220. TMC435 at 45 mg/kg/day suppressed the levels of total CYP450 and CYP1A (male and female), CYP2B (males), CYP3A (males and females), CYP2E (males and females), CYP4A (females), and thyroxine UGT (male and female). CYP4A was induced in male dogs and showed an inverse relationship with dose with the greatest effect seen at the low dose. CYP1A also appeared to be induced in females at the low dose however there was not a clear dose dependency and so it is not known if this is a true test article related effect or not. Overall the enzyme effects were greater in male dogs vs. female dogs.

Figure 2 Proposed In Vivo Biotransformation Pathways of TMC435 from Radiolabeled Studies in Rat, Dog and Human



Radiolabeled Metabolism and Excretion Studies:

Human: The human radiolabeled ADME study (Study NC219) is reviewed in detail in the Clinical Pharmacology review. In human plasma, M21 was the most abundant circulating metabolite (7.96% of mean plasma AUC_{0-24h} of the parent). Similar to excretion in nonclinical species, TMC435 was mainly excreted in feces (55.6% up to 95.2%) with low amounts in urine (0.009%-0.139%).

Rat: The metabolism and excretion of radiolabeled ¹⁴C-TMC435350 in the rat was studied after a single oral dose at 120 mg/kg (Study NC169). Almost the entire dose was excreted in feces (98.7-99%) with very little in urine (0.01-0.02%) similar to human. Unchanged parent was the main component in plasma (T_{max} 4 h) and feces (84.2-95.3%). Metabolite 18 formed by O-demethylation of the parent was the major circulating plasma metabolite with a T_{max} of 4-7 hours. The parent drug was oxidized to Metabolites 21 (the most abundant circulating human metabolite) and 22. Some metabolites were only detected by a more sensitive method of LC/MS-MS and included Metabolites 27(oxidation of the parent to the macrocycle), 23, 24, and 25 (mono-oxidation of the macrocycle and di-oxidation of the aromatic substructure).

Dog: The metabolism and excretion of radiolabeled ¹⁴C-TMC435350 in the dog was studied after a single oral dose at 30 mg/kg (Study No. TMC435-NC212 (FK6498)). Similar to the rat the majority of the parent was excreted in the feces (96%) with very

little excreted in the urine (0.09%). The parent was the main circulating plasma moiety at all timepoints and was also the main component in feces (52.06% of the dose). Three circulating metabolites were identified in the plasma and included Metabolites 8, 18 and 21. The major metabolic pathway was O-demethylation of the parent to Metabolite 18 (18.76% of the dose). Oxidation of Metabolite 18 formed Metabolite 14 (1.94% of dose) or Metabolite 8 (0.42% of dose, O-demethylation and oxidation at the aromatic moiety). Metabolite 21 (3.27% of dose) was formed by oxidation of the macrocyclic moiety. Other clearance pathways led to the formation of Metabolites 16 (2.05% of dose) and 11 (1.28% of dose). Additional Metabolites included 22, 27, 24, 25 and 26 which could be only detected by LC/MS-MS.

EXCRETION

The biliary excretion of TMC435 was evaluated in bile duct cannulated rats after a single oral dose of 40 mg/kg (Study No. NC 194/FK6047). Large concentrations of unchanged drug were found in the bile at 0-4 hours and 4-8 hours. The in vivo metabolites found in the bile were formed by oxidation, demethylation and glucuronidation with the highest levels observed at 0-4 hours and 4-8 hours.

In Vitro and In Vivo Studies of TMC435 and Membrane Transporters

TMC435 is a transported substrate of OATP1B1, OATP1B3, and OATP2B1 (Study No. FK10099). In a study in wild-type and knock-out Oatp1a/1b mice, OATP1A/OATP1B transporters played a significant role in the PK of TMC435 (Study FK10098) with 10-fold higher plasma exposures in KO mice vs. wild type mice and lower liver concentrations in KO mice. TMC435 is an inhibitor of taurocholate (BSEP) and estradiol 17B-D-glucuronide (MRP2) hepatic uptake in rat and human hepatocyte suspension cultures in vitro (Study No. NC241); BSEP - IC₅₀ 1.67 μM and MRP2 - IC₅₀ 6.4-19.1 μM (Study NC242). Inhibition experiments with ritonavir, rifampicin and cyclosporine suggest active hepatic transport contributes to the saturable component of hepatic uptake (Study FK6806).

TMC435 is a potent substrate of the 3 major ABC transporters – ABCB1, ABCC2, and murine Abcg2 (Study NC239). Based on these data the sponsor concludes that TMC435 would not likely distribute to the brain, testes, or fetal tissues at significant levels. In this study, it was also determined that ritonavir is not an efficient inhibitor of the ABC transporters.

5.2 TOXICOKINETICS

TK analyses are included in the general toxicology study reviews in Section 6.

6 GENERAL TOXICOLOGY

6.1 SINGLE-DOSE TOXICITY

Single dose oral toxicity studies in the rat and dog were previously reviewed by Christopher Ellis, PhD. In the rat single-dose toxicity study (NC140) at doses of 0, 50, 200, and 1000 mg/kg/day, the observations were limited to an increased incidence of soft feces at 200 and 1000 mg/kg/day. In the dog single-dose toxicity study (NC145) at doses of 0, 10, 40, 160 and 320 mg/kg/day, the findings included reductions in

cholesterol and bilirubin as well as pale feces at 320 mg/kg/day. The single-dose study in the dog was followed by a 5-day repeat dose study at 160 mg/kg. Findings in the 5 day repeat dose portion included increases in salivation, pale feces, vomiting, bilirubin, AST, ALT, neutrophils, WBCs, and decreases in body weight, cholesterol, triglycerides as well as minimal hepatocellular necrosis.

6.2 REPEAT-DOSE TOXICITY

Repeat-dose toxicity studies of TMC435 were conducted in the mouse, rat, dog and monkey by the oral route (gavage or dietary feed). The pivotal chronic toxicity studies were conducted in the rat and dog based on the similar PK and metabolite profiles compared with human. The 2-week rat (NC144), 1-month rat (NC177), 6-month rat with 3-month interim kill (NC179), 2-week dog (NC161), 1-month dog (NC175), and 28-day monkey (NC181) toxicity studies were reviewed by Christopher Ellis, PhD and are included in the appendices. Full reviews of the 3-month mouse dietary feed study (NC253), the 3-month rat dietary feed study (NC244), the 6-month oral gavage dog study (NC226), and the 39-week oral gavage dog study (NC207) are included in this section. The dietary feed studies in the rat and mouse were evaluated in order to understand the toxicity of TMC435 in the absence of potential gavage or formulation related effects. In addition, the exposures were slightly higher in the dietary feed studies compared with the oral gavage studies; therefore, higher exposures would increase the likelihood of fully characterizing the toxicity of TMC435. A summary of the toxicity findings by species is included below.

Mice

In the 14-day mouse oral gavage study (NC205) the NOAEL was 150 mg/kg/day (mean AUC 151.5 $\mu\text{g}\cdot\text{h}/\text{ml}$, 2.6X the clinical AUC). Toxicities included minimal centrilobular hepatocellular hypertrophy and/or dense cytoplasmic staining and prominent mitosis observed in liver, small increase in liver weight, decrease in cholesterol, increase in total bilirubin and ALT, and minimal swelling/vacuolization of apical enterocytes of duodenum. In the 3-month dietary feed studies, doses of 0.5, 2 and 5 g/kg/day (NC233), and 3 g/kg/day (NC253) were evaluated. These studies were conducted to determine dose levels for potential mouse carcinogenicity studies. The dietary feed studies were reviewed because higher exposures were achieved in these studies compared with the oral gavage toxicity studies. Additionally, any potential gavage or formulation related issues could be avoided by dietary feed administration. In Study NC233, at doses of 0.5, 2 and 5 g/kg/day, the 5 g/kg/day group was terminated early on Day 4/5 due to rapid deterioration and moribundity/mortality. The findings at the 0.5 and 2 g/kg/day dose groups were consistent with the findings observed at 3 g/kg/day in Study No. NC253 but higher exposures were achieved at 3 g/kg/day. Therefore, Study NC233 was reviewed only briefly and Study NC253 was formally reviewed in order to evaluate the potential toxicity of TMC435 at higher exposures. In the 3-month dietary feed study at the dose of 3 g/kg/day, adverse clinical signs indicative of morbidity were observed towards the end of the study, significant body weight decreases were seen throughout the study with the greatest decrease at Week 1 (155% in males and 113% in females). Hematology changes included a decrease in hematocrit, hemoglobin, mean cell hemoglobin, and mean cell volume as well as an increase in lymphocytes (1.46-fold

increase). Target organs included the liver (minimal to slight hepatocyte hypertrophy, increased ALP, ALT and/or AST, and bilirubin); pancreas (minimal to slight decreased acinar cell zymogen/basophilia, acinar cell vacuolation in one male, increased lipase and amylase); duodenum (minimal vacuolation of the apical enterocytes), and adrenal glands (reduced incidence of X zone vacuolation and increased incidence of minimal prominent X zone (5/10) and cortical atrophy (7/10) in females). The NOAEL in the 3-month mouse dietary feed study was 0.5 g/kg/day (mean AUC 33.3 $\mu\text{g}\cdot\text{h}/\text{ml}$, 0.6X the clinical AUC).

Rats

Based on 2-week, 1-month, 3-month and 6-month oral gavage toxicity studies the NOAEL is 120-150 mg/kg/day in rats (Mean AUC 25.2 $\mu\text{g}\cdot\text{h}/\text{ml}$ in the 2-week to 26.9 $\mu\text{g}\cdot\text{h}/\text{ml}$ in the 6-month, 0.4-0.5X the clinical AUC). Toxicities in the rat included changes in liver-related parameters (reduced cholesterol, total protein and increased ALT), urine characteristics (pH, color and presence of occult blood), and hematology (reduction in reticulocytes and increase in neutrophils). Although no recovery animals were included in the 6 month rat study, similar test article related observations were noted in the 1 month rat toxicology study and these findings were not present or showed a trend towards reversibility following a 1 month recovery period (NC177); therefore, the absence of a recovery group in the 6-month rat toxicity study is not a deficiency.

In the 3-month rat dietary feed study (NC244), ~2X higher AUC was achieved in females dosed at 2 g/kg/day (AUC_{0-24h} 99345 ng $\cdot\text{h}/\text{ml}$) compared with the 6-month rat oral gavage study (AUC 52700 ng $\cdot\text{h}/\text{ml}$). Therefore, the rat dietary feed study was evaluated to characterize the toxicity of TMC435 in the rat at higher exposures. In the 3-month rat dietary feed study, findings included significant body weight decrease across dose groups as low as 40% in males and 32% in females at the 2 g/kg/day high dose. Decreased reticulocytes in males (up to 23.8% at high dose), increased platelets (up to 25% at high dose), and a slight increase in APTT (3.7% at high dose) were observed. Significant elevations in ALP, ALT, AST, bile acids, lipase, urea and phosphorus as well as decreased amylase, triglycerides, potassium and total protein. Histopathology in the liver (minimal centrilobular hypertrophy) and small intestine/jejunum, minimal to slight vacuolation of the apical enterocytes were observed. There was no NOAEL identified in the study based on the adverse body weight decreases across dose groups; the sponsor proposed an MTD of 500 mg/kg/day for the study (Mean AUC at 500 mg/kg/day – 40.8 $\mu\text{g}\cdot\text{h}/\text{ml}$, 0.7X the clinical AUC).

Dogs

In dogs, toxicity observations in the 2-week oral gavage toxicity study and the 1-month oral gavage toxicity study with 1-month recovery included changes in urine characteristics (moderate to high levels of bilirubin, presence of blood, high turbidity and changes in color), clinical observations (reductions in body weight and food consumption and increased hypersalivation and soft, mucoid/hemorrhagic, pale feces), clinical chemistry (moderate reduction in cholesterol and total protein and increases in total and direct bilirubin and ALT), hematology (reduction in reticulocytes correlating with small reductions in RBC, hemoglobin and hematocrit), and gross and

histopathological changes (minimal to slight multi-focal hepatocyte necrosis). Additionally, in the 2-week oral gavage toxicity study at doses up to 120 mg/kg/day in females and 160 mg/kg/day in males, minimal liver cholestasis, arteriopathy, as well as potential cardiac toxicity (acute endocardial and myocardial necrosis) were observed. Higher exposures were achieved in this study compared with the longer duration chronic dog toxicity studies. The NOAEL in the 2-week study was 10 mg/kg/day (mean AUC at 10 mg/kg – 73 $\mu\text{g}\cdot\text{h}/\text{ml}$, 1.3X the clinical AUC). The NOAEL In the 1-month study was also 10 mg/kg/day although the mean AUC was lower (Mean AUC at 10 mg/kg – 17 $\mu\text{g}\cdot\text{h}/\text{ml}$, 0.3X the clinical AUC). In the 1-month dog oral gavage toxicity study with 1-month recovery, no test-article related changes were noted in the 1 month recovery group except ALT (92%) and urine color (67-100%) were still increased in female, and male and female dogs, respectively.

The chronic 6-month oral gavage and 9-month oral gavage dog toxicity studies overall showed consistent toxicities with the shorter duration 2-week and 1-month studies. The NOAEL in the 6-month dog study was 15 mg/kg (mean AUC at 15 mg/kg/day – 74.8 $\mu\text{g}\cdot\text{h}/\text{ml}$; 1.3X multiple over clinical AUC). The NOAEL in the 9-month dog study was 5 mg/kg/day based on a female at the 15 mg/kg/day dose group that was sacrificed moribund; the sponsor attributed this female's death to a gavage error; however, this female also had extremely high plasma, heart and liver concentrations (mean AUC at 5 mg/kg/day – 6.8 $\mu\text{g}\cdot\text{h}/\text{ml}$; 0.1X multiple over clinical AUC). Therefore, it is not known if TMC435 played a direct role in this animal's death. No cardiotoxicity was observed in the chronic toxicity studies. Interestingly, the females in the 6-month study showed 5-6X higher AUC values than females in the longer duration 9 month study. Male exposures were similar. The main target organs were the liver (hepatocellular necrosis associated with hemorrhage – only in the 6 month dog study, neutrophils, macrophages, increases in ALT) and small intestine (dilatation of the duodenum crypts and lacteals, vacuolization of apical enterocytes of the jejunum and duodenum that correlated with an increase in fat droplets, and congestion). Testicular and epididymal findings were seen in both chronic dog studies that included vacuolization of the epididymides and testicular tubular atrophy/hypoplasia; these findings were also seen in control animals at slightly lower incidences making it difficult to determine the relationship of these findings to treatment.

Monkey

No NOAEL was determined in the Rhesus monkey study due to only one treatment group evaluated in the study. Toxicity observations included: feces discoloration (orange/red/dark red/brown) with presence of blood on one occasion, slight reductions in RBC, HCT & PCV%, increases in total and direct bilirubin and AST, a decrease in cholesterol, traces of blood detected in urine, and some histopathology changes. Toxicity observations in the 14-day repeat dose (20 mg/kg/day) portion of the study were limited to hypersalivation, emesis, and increased AST.

In conclusion, the major toxicities observed with TMC435 (hematology/chemistry changes, liver toxicity and small intestine changes) were consistent across species.

Study title: TMC435350: Supplementary Toxicity Study by Dietary Administration to CD-1 Mice for 13 weeks

Study no.: TMC435-TiDP16-NC253/TOX9258
 Study report location: Tibotec Pharmaceuticals Ltd.,
 A member of the Johnson and Johnson
 group of companies,
 Little Island, Co Cork, Ireland
 Conducting laboratory and location:  (b) (4)

Date of study initiation: January 14, 2009
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #s, and % purities:

08H19/F017	97.6% (w/w)
08H20/F017	97.7 % (w/w)
08H21/F017	97.6% (w/w)
08I03/F017	96.9% (w/w)
08I02/F017	98.2 % (w/w)

Key Study Findings

- **Adverse clinical signs indicative of morbidity observed towards the end of the study:** hunched posture, underactivity, thin build, pallor, abdominal distension, piloerection and reduced body temperature.
- **Significant body weight decreases** throughout study; at Week 1 – body weight decrease of 155% in males and 113% in females.
- **Hematology** - Decrease in hematocrit, hemoglobin, mean cell hemoglobin, and mean cell volume. Increase in lymphocytes (1.46-fold increase).
- **Liver** – minimal to slight hepatocyte hypertrophy, increase in ALP, ALT and/or AST, and bilirubin.
- **Pancreas** - minimal to slight decreased acinar cell zymogen/basophilia, acinar cell vacuolation in one male, increased lipase and amylase associated with pancreas histopathology.
- **Duodenum** - minimal vacuolation of the apical enterocytes.
- **Forestomach (nonglandular stomach)** - minimal to slight hyperkeratosis in 2/10 males and 3/10 females including one control female
- **Adrenal** - reduced incidence of X zone vacuolation and increased incidence of minimal prominent X zone (5/10) and cortical atrophy (7/10) in females.
- **No NOAEL** based on adverse clinical signs and body weight decreases. In the mouse dietary feed study (NC233) at doses of 0.5, 2 and 5 mg/kg/day, the high dose was terminated on Day 4/5 due to high moribundity/mortality; a no effect level was not established in the study.

Methods

Doses: Vehicle, 3 g eq/kg/day

Frequency of dosing: Daily
Route of administration: Dietary feed
Dose volume: N/A
Formulation/Vehicle: Test article mixed with feed at a concentration of 40000 ppm, salt to base conversion of 1.03.
Species/Strain: Mouse/CD-1
Number/Sex/Group: 10/sex/group
Age: 33 to 39 days
Weight: Males – 24.8-32.1 g, Females – 20.9-28.9 g
Satellite groups: TK
Unique study design: None.
Deviation from study protocol: None reported.

Observations and Results

Test Article Intake

The calculated test article intake was calculated weekly and was averaged over the 13 week treatment period of the study closely approximated the nominal dose of 3 g eq/kg/day. The calculated test article intake in males was 2.98 g eq/kg/day and in females was 3.20 g eq/kg/day.

Mortality

One female satellite mouse showed a rapid deterioration of condition and was sacrificed at Week 11. Clinical signs in this animal included reduced body temperature, shallow breathing, partially closed eyes (dark in color), hunched posture, piloerection, pallor of the whole body, underactivity and thin build. Macroscopic findings in this animal included distension of the GI tract, a few dark corpus depressions in the mucosa of the stomach, patchy congestion of the lungs and dark adrenals. The death was considered to be related to treatment.

Clinical Signs

Mice were inspected twice daily for reactions to test article and ill-health. Cage inspections were performed once daily. Detailed observations were performed once daily for the first week, twice per week from Weeks 2-4, and weekly thereafter. Physical examinations were performed weekly.

Test article related clinical signs were seen in 4 males (1 main study and 3 satellites) and 5 females (2 main study and 3 satellites) mainly towards the end of the study at Weeks 10-13. These clinical signs included (in order of the most frequently observed): hunched posture, underactivity, thin build, pallor, abdominal distension, piloerection and reduced body temperature. Yellow discoloration of the pinna was seen in two males.

Body Weights

Body weights were measured on Day -7, Day -4, Day 0 (1st dose), weekly during the dosing period, and prior to necropsy. A decrease in body weight gain and absolute body weight were seen in males and females. The body weight gain decrease from Week 0-1 Week 1 was -155% in males and -113% in females compared to controls. Body weight

gains were similar for females for the remainder of the study. For males, body weight gains were similar from Week 2 to 8 and with further body weight decreases observed from Week 8 to 13. The overall body weight gain decrease from Week 0 to Week 13 was -43% in males and -31% in females compared with controls. The absolute body weight decrease at Week 13 was -19% in males and -14% in females.

Feed Consumption

Food consumption was measured weekly and was decreased in treated males (-11%) and females (-17%) from Week 1 to Week 13 compared to controls.

Ophthalmoscopy

Not applicable.

ECG

Not applicable.

Hematology

Hematology was measured during Week 13 (blood samples collected by retro-orbital sinus). In females, decreases in hematocrit, hemoglobin, mean cell hemoglobin, and mean cell volume were seen. In males, hemoglobin concentration was slightly decreased (6%). Total white blood cell or leucocyte counts were increased in females by 1.41-fold; the increase in total white blood cells appeared to be attributable to an increase in lymphocytes (1.46-fold increase). A summary of the notable hematology differences is included in the sponsor's table below (Table 4).

Table 4 Summary of Notable Hematology Changes in Male and Female Mice Following Dietary Feed of TMC435 at 3 g/kg/day for 13 week

Dose group g eq./kg/day	1M	2M	1F	2F
	0	3	0	3
	Means		Means	
Haematocrit L/L	-	-	0.458	0.96X*
Haemoglobin conc. g/dL	14.3	0.94X	14.9	0.92X**
Mean cell haemoglobin pg	-	-	16.0	0.91X**
Mean cell haemoglobin conc. g/dL	-	-	32.6	0.96X**
Mean cell volume fL	-	-	49.1	0.95**
Total leucocyte count x10 ⁹ /L	7.51	1.04X	4.56	1.41X*
Lymphocyte count x10 ⁹ /L	5.66	1.07X	3.58	1.46X*

Control columns show actual means, Group 2 show fold differences from control (X control).

Statistical significance: *p<0.05; **p<0.01

Clinical Chemistry

Clinical chemistry parameters were assessed during Week 13. Clinical chemistry changes included increases in mean values for males and females in: ALP, ALT, AST (females only), lipase activity, bilirubin, as well as decreases in creatinine, glucose, cholesterol, and triglycerides. Slight increases in sodium, chloride and a substantial increase in phosphorus (1.47-1.51-fold) were observed. Total protein, albumin (males only), α 1-globulin, β -globulin (females only), and γ -globulin (females only) were reduced.

The albumin:globulin ratio was slightly increased (1.12-fold) from controls. On an individual animal basis, GGT was high in one male and one female, ALP, ALT and/or AST, were high in a few animals and BILIRUBIN was high in the majority of females and 6 out of 10 males. Increased lipase in one male and one female mouse was associated with increases in amylase as well as pancreas histopathology. In the female, histopathologic examination of the pancreas showed a slight decrease in zymogen/basophilia in the acinar cells and in the male acinar cell vacuolation was seen. A summary of the notable clinical chemistry changes is summarized in the sponsor's table (Table 5) below.

Table 5 Summary of Notable Clinical Chemistry Changes in Male and Female Mice Following Dietary Feed of TMC435 at 3 g/kg/day for 13 weeks

Dose group g eq./kg/day	1M	2M	1F	2F
	0	3	0	3
	Means		Means	
ALP (U/L)	64	3.3X	57	1.49X**
ALT (U/L)	51	1.37X	40	1.98X
AST (U/L)	-	-	65	1.32X
Lipase (U/L)	32	1.63X*	31	1.61X*
Bilirubin (µmol/L)	2	4.5X**	2	5.5X**
Creatinine (µmol/L)	10	0.7X*	11	0.64X*
Glucose (mmol/L)	13.36	0.65X**	12.97	0.78X**
Cholesterol (mmol/L)	3.27	0.20X**	2.41	0.23X**
Triglycerides (mmol/L)	1.49	0.30X**	1.65	0.34X**
Sodium (mmol/L)	151	1.02X**	149	1.02X**
Chloride (mmol/L)	111	1.02X*	111	1.02X
Phosphorus (mmol/L)	1.54	1.47X**	1.62	1.51X**
Total protein (g/L)	57	0.89X**	55	0.95X**
Albumin (g/L)	35	0.86X**	-	-
a1 (g/L)	5	0.80X**	3	0.67X**
Beta (g/L)	-	-	11	0.91X*
Gamma (g/L)	-	-	2	0.5X*
Albumin: globulin ratio	-	-	1.62	1.12X*

Control columns show actual means, Group 2 show fold differences from control (X control).

Statistical significance: *p<0.05; **p<0.01

Urinalysis

Urinalysis samples not collected.

Gross Pathology

Macroscopic findings at 13 weeks in the mouse showed roughening of the forestomach epithelium in male (5/10) and female (7/10) mice, depressions in the oesophageal groove on the forestomach of females (3/10), and distension of the small intestine and/or cecum in males (7/10) and females (8/10).

One treated male had a confirmed mass in the lumbar skeletal muscle that was diagnosed to be an osteosarcoma. The sponsor did not consider the finding to be treatment related.

The dark appearance of the adrenals in some males and females was not considered toxicologically relevant because there were no relevant histological changes associated.

Organ Weights

Organ weight changes were seen in females. Liver weights were increased in females (1.05X – mean absolute change, 1.19X body weight normalized) compared to controls. Heart weights (0.82X – absolute, 0.70X – body weight adjusted) and ovary weights (0.48X) were decreased in females. The decrease in ovary weights may be related to the decrease in corpora lutea.

Histopathology

Adequate Battery

Yes.

Peer Review

Yes.

Histological Findings

Test article related histopathology findings were seen in the liver, pancreas, stomach, duodenum, adrenals and ovaries. In the liver, minimal to slight hepatocyte hypertrophy was seen in males (10/10) and females (9/10). In the pancreas, minimal to slight decreased zymogen/basophilia was seen in the acinar cells in males (6/10) and females (1/10). Acinar cell vacuolation was seen in one male. In the stomach, minimal to slight hyperkeratosis of the nonglandular portion of the stomach was seen in 2/10 males and 3/10 females including one control female. Reduced corpora lutea was observed in 4/10 females. One high dose male was confirmed by histopathology to have an osteosarcoma, this was the same male that presented with a mass in the lumbar skeletal muscle.

In the duodenum, minimal vacuolation of the apical enterocytes was seen in 2/10 males and 3/10 females. Finally, a reduced incidence of X zone vacuolation was seen in treated females and an increased incidence of minimal prominent X zone (5/10) and cortical atrophy (7/10) were seen in female animals.

The sponsor considered the majority of the findings to be incidental. The adrenal findings were attributed to delayed sexual maturation. The sparse corpora lutea were attributed to the body weight loss and possibly delayed sexual maturation.

Special Evaluation

None.

Toxicokinetics

TK was evaluated on Day 3 and Day 87. The TK following repeat dietary feed exposure showed reduced AUC (Males 33%, Females 54%) and C_{max} (Males – 11%, Females – 51%) exposures on Day 87 compared to Day 3; reduced exposure following repeat dosing was observed. The TK parameters are summarized in the sponsor's table below (Table 6).

Table 6 Summary of Toxicokinetic Parameters in Male and Female Mice Following Dietary Feed of TMC435 at 3 g/kg/day for 13 weeks

Treatment Gender Day	Group 2 (3 g eq./kg/day)			
	Male		Female	
	3	87	3	87
C _{max} (ng/ml)	13100	11653	18400	9077
AUC _{0-24h} (ng.h / ml)	283032	189244	311499	143459

Study title: TMC435350: Supplementary Toxicity Study by Dietary Administration to CD Rats for 13 weeksStudy no.: **TMC435-TiDP16-NC244/ TOX9170**Study report location: Tibotec Pharmaceuticals Ltd.,
A member of the Johnson and Johnson
group of companies,
Little Island, Co Cork, Ireland

Conducting laboratory and location:



Date of study initiation: 14 April 2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: TMC435350.Na, Lot Nos 09C23/F017,
Purity 97.5% (w/w), 09C24/F017 Purity
97.6% (w/w), 09C25/F017 Purity
98.3% (w/w), 09C26/F017 Purity 98.3%
(w/w)**Key Study Findings**

- Significant body weight decrease across dose groups as low as 40% in males and 32% in females at the high dose.
- Decreased reticulocytes in males (up to 23.8% at high dose), increased platelets (up to 25% at high dose), increased APTT (3.7% at high dose).
- Significant elevations in ALP, ALT, AST, bile acids, lipase, urea and phosphorus. Decreased amylase, triglycerides, potassium and total protein.
- Liver - minimal centrilobular hypertrophy
- GI - jejunum, minimal to slight vacuolation of the apical enterocytes
- No NOAEL based on adverse body weight decrease (>10%) in all dose groups; sponsor proposed MTD – 500 mg/kg/day.

Methods

Doses: 0, 500, 1000, 2000 mg/kg/day

Frequency of dosing: Daily

Route of administration: Oral, dietary feed

Dose volume: NA
 Formulation/Vehicle: TMC435 was incorporated directly into the basic diet/the vehicle was the feed alone (Rat and Mouse No. 1 Maintenance Diet)
 Species/Strain: Rat/ CrI:CD® (SD)IGS BR strain
 Number/Sex/Group: 10/sex/group
 Age: 46 days or less
 Weight: 180 to 246 g for males and 159 to 209 g for females
 Satellite groups: TK – 3/sex/group
 Unique study design: None.
 Deviation from study protocol: None reported.

Observations and Results

Mortality

No mortality was observed.

Clinical Signs

Rats were inspected twice daily for reactions to test article and ill-health. Cage inspections were performed once daily. Detailed observations were performed once daily for the first week, twice per week from Weeks 2-4, and weekly thereafter. Physical examinations were performed weekly. Clinical signs included pale feces at 500 (starting at Week 11), 1000 (starting at Week 8) and 2000 (starting at Week 4) mg/kg/day. Brown staining was seen on the dorsal body surface, head, muzzle, pinna and/or tail and started at Week 5 or 6 and was most prevalent in the last 4 weeks of treatment.

Body Weights

Body weights were measured pre-dose (Day -7 and Day -4), on the study day start, weekly during treatment and prior to necropsy. Statistically significant decreases in body weights (as low as 40% in high dose males and 32% in high dose females) were seen across dose groups for males and females. A summary of the body weight gain changes from Week 0 to 13 is provided in the sponsor's table below (Table 7).

Table 7 Summary of Rat Body Weight Gain Change from Week 0 to Week 13 following Dietary Feed Administration of TMC435

Group/sex Dose eq./kg/day	1M 0	2M 500	3M 1000	4M 2000	1F 0	2F 500	3F 1000	4F 2000
Body weight gain Week 0-13	325g	249g**	232g**	194g**	137g	113g**	97g**	93g**
X Control	-	0.76	0.71	0.60	-	0.82	0.71	0.68

Statistical-significance: ** P<0.01

Achieved Doses

The mean achieved intake of TMC435 from Week 0 to Week 13 approximated the nominal doses. The food conversion efficiency was 512, 1009, 2009 mg/kg/day for males and 507, 1003 and 1999 mg/kg/day for females.

Feed Consumption

Food consumption was measured at Week -1 and weekly throughout the study. Decreased food consumption was seen across dose groups with the greatest decrease observed during the first week of dosing. Food consumption decreases were 13% at the low dose, 16% at the mid dose and 21% at the high dose in males and 13% at the low dose, 19% at the mid-dose and 16% at the high dose in females.

Ophthalmoscopy

Ophthalmic examinations were evaluated pre-dose and during Week 13 using a binocular indirect ophthalmoscope and a slit-lamp biomicroscope. There were no test article related ophthalmic findings.

ECG

Not applicable.

Hematology

Hematology was evaluated at Week 13. Hematological changes in male mice included a slight increase in red blood cells (2.3-3.4% at the mid and high dose) and a statistically significant reduction in reticulocytes from controls (-17.1% at the mid dose and 23.8% at the high dose). No significant treatment related hematology changes were observed in females.

Coagulation

Coagulation was evaluated at Week 13. In females, statistically significant increases in platelet numbers were seen at the mid-dose (19.2%) and high dose (25.2%) groups. A statistically significant increase in prothrombin time (7.9%) was seen in high dose females. Although not statistically significant, a slight increase in activated partial thromboplastin time (APTT) of 3.7% was seen in high dose females.

Clinical Chemistry

Clinical chemistry was evaluated at Week 13. Clinical chemistry changes in males included statistically significant elevations at the mid (30%) and high (20%) dose groups in ALP, ALT, and AST. Decreases in amylase (up to 22%), and triglycerides (as low as 42%), as well as increases in lipase (up to 10%), bile acids (up to 2-fold) and urea (up to 40%) were seen. Most of these changes were statistically significant with the exception of the increase in bile acids. Increases in phosphorus (20%) and decreases in potassium (15%) were also seen.

In females, decreases in amylase (as low as 13%, not statistically significant), increases in lipase (up to 20%), increase in bile acids (up to 65%, not statistically significant), as well as an increase in phosphorus (up to 20%), and a decrease in total protein (as low as 5%) were seen.

The clinical chemistry changes in males and females following 13 weeks of treatment are summarized in the sponsor's table below.

Table 8 Summary of Notable Clinical Chemistry Changes in Male and Female Rats Following Dietary Feed of TMC435 for 13 weeks

Dose group	1M	2M	3M	4M	1F	2F	3F	4F
mg eq./kg/day	0	500	1000	2000	0	500	1000	2000
	Mean				Mean			
	<i>s</i>				<i>s</i>			
ALP (U/L)	76	1.0X	1.3X**	1.2X**	-	-	-	-
ALT (U/L)	33	1.1X	1.2X**	1.3X**	-	-	-	-
AST (U/L)	74	1.1X*	1.2X**	1.3X**	-	-	-	-
Amylase (U/L)	2442	0.97X	0.86X*	0.78X**	1690	1.13X	0.85X	0.87X
Lipase (U/L)	10	1.0X	1.1X*	1.1X**	11	1.0X*	1.0X*	1.2X**
Bile Acids (μ mol/L)	38.0	0.52X	0.69X	2.01X	25.6	1.03X	1.13X	1.65X
Urea (mmol/L)	4.52	1.3X*	1.1X*	1.4X**	-	-	-	-
Triglycerides (mmol/L)	1.17	0.65X**	0.74X**	0.58X**	-	-	-	-
Phosphorous (mmol/L)	1.91	1.2X**	1.2X**	1.2X**	1.61	1.1X*	1.2X**	1.2X**
Potassium (mmol/L)	4.8	0.96X	0.94X*	0.85X**	-	-	-	-
Total protein (g/L)	-	-	-	-	74	0.97X	0.96X	0.95X**

Control columns show actual means; Groups 2, 3 and 4 show fold differences from control means (X Control).

Statistical-significance: * P<0.05; ** P<0.01

Urinalysis

Urinalysis was evaluated at Week 13. Urinalysis findings included a statistically significant increase in urine volume in high dose males (40.8%) and a decrease in urinary protein in mid-dose (-21.4%) and high (-36.7%) dose males.

Gross Pathology

Gross pathology findings were observed in the lung and GI tract. Abnormal contents in the GI tract were observed for almost all male animals (9/10 at low dose and 10/10 at each of the mid- and high-dose groups) as well as the majority of female animals (4/10 at low-dose, 7/10 at mid-dose, and 9/10 at high dose). Macroscopic lung findings included areas of congestion in 3/10 males at the mid-dose and 2/10 females at the low-dose group and 1/10 females at each of the mid- and high-dose groups. Pale areas in the lungs of female animals were seen at an incidence greater (2/10-low dose, 6/10-mid dose, 5/10 high dose) than control females (1/10 - controls). There were also lung adhesions seen in one mid-dose female. The sponsor did not consider any of the gross pathology findings to be treatment related including the lung findings because there were no relevant lung histopathology findings that were associated with the gross findings.

Organ Weights

No treatment-related significant organ weight changes were seen.

Histopathology

Adequate Battery

Yes.

Peer Review

Yes.

Histological Findings

Test article related histopathology findings were seen in the liver and the jejunum. In the liver, minimal centrilobular hypertrophy was seen in males (4/10 at low-dose, 2/10 at mid-dose, and 7/10 at high-dose) and females (3/10 at low-dose, 2/10 at mid-dose, and 6/10 at high-dose) across dose groups with no findings in controls. In the jejunum, minimal to slight vacuolation of the apical enterocytes was seen in males (2/10 at low-dose, 1/10 at mid-dose, and 4/10 at high-dose) and females (1/10 controls, 2/10 at low-dose, 3/10 at mid-dose, and 6/10 at high-dose) across dose groups including one control female. There were a number of lung findings but no clear test article relationship since many of the findings were also seen in controls. The findings observed in the lungs and bronchi included aggregations of alveolar macrophages, alveolar congestion/hemorrhage, alveolar epithelial hyperplasia, alveolar edema, alveolar osseous metaplasia, alveolitis, perivascular inflammatory cells, perivascular lymphoid aggregates.

Special Evaluation

None. Heart samples were prepared for potential future electron microscopy analyses.

Toxicokinetics

Toxicokinetics were evaluated at Week 4 and Week 13. The TK parameters are summarized in the sponsor's table below (Table 9). C_{max} and AUC increased with dose less than dose proportionally. Exposures were higher in females vs. males for C_{max} (45-65%) and AUC (62-90% or almost 2-fold higher). Slight accumulation (1.2-2.1 fold for C_{max} and 1.1-1.2-fold for AUC) was seen in females between Week 4 and Week 13. No accumulation was seen in males between Week 4 and Week 13. In fact, a decrease in exposure of 45% was seen in high dose males between Week 4 and Week 13.

Table 9 Summary of Toxicokinetic Parameters in Male and Female Rats Following Dietary Feed of TMC435 for 13 weeks

Sex	Male			Female		
Dose (mg eq./kg/day)	500	1000	2000	500	1000	2000
Week	4					
C_{max} (ng/ml)	1633	1727	3270	2687	2497	5223
AUC _{0-24h} (ng.h/ml)	26316	30340	48751	47848	49162	92746
Week	13					
C_{max} (ng/ml)	1303	1667	1620	5673	3767	6247
AUC _{0-24h} (ng.h/ml) ¹⁾	23756	28264	26840	57757	55395	99345

Dietary Feed Analysis

Dietary feed samples were analyzed at Week 1, 7 and 13. All of the samples met the acceptance criteria specified in the protocol. The individual diet samples contained 93-107% of the nominal concentration of TMC435.

Study title: 6-month repeated dose oral toxicity study of TMC435350 in the beagle dog

Study no.: **TMC435350-TiDP16-NC226/TOX8556**
 Study report location: Tibotec Pharmaceuticals Ltd.
 a member of the J&J group of companies
 Eastgate Village
 Eastgate
 Little Island, Co Cork Ireland
 Conducting laboratory and location: Global Preclinical Development, Beerse
 site, Turnhoutseweg 30
 2340 Beerse, Belgium
 Date of study initiation: Sep 28, 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: TMC435350, lot no. ZR494617PFA061
 (conversion factor = 1.03), purity 96.7%

Key Study Findings

- Clinical signs: soft, pale and mucoid feces, salivation, vomiting,
- Decrease in APTT, increase in fibrinogen, reticulocyte decrease.
- Decrease in triglycerides, cholesterol. Increase in total bilirubin, ALT, AST, and ALP. Increases in urobilinogen and bilirubin in urine.
- **Adrenals** - swollen zona fasciculata cells (Grade 1) in 1/4 mid dose and 1/4 high dose males and vacuolization in 1/4 controls, 2/4 low dose, and 2/4 mid-dose females.
- **Liver** – multifocal nonzonal hepatocellular necrosis associated with hemorrhage, neutrophils and correlated with increases in ALT and liver weight increase.
- **Small intestine** – dilatation of the duodenum crypts and lacteals, vacuolization of apical enterocytes of the jejunum and duodenum, correlated with an increase in fat droplets.
- **Testes/epididymides** - vacuolization of the epididymides and testicular atrophy/tubular hypoplasia in 1/4 control animals, 2/4 low dose, and 2/4 high dose animals (Grade 1 and Grade 2 severity scores).
- **NOAEL - 15 mg/kg based on adverse liver toxicity at 45 mg/kg/day** (male AUC at 15 mg/kg/day – 69.9 µg*h/ml, female AUC at 15 mg/kg/day – 79.7 µg*h/ml, mean – 74.8 µg*h/ml; 1.3X multiple over clinical AUC).

Methods

Doses: 5, 15 or 45 mg eq./kg/day
 Frequency of dosing: Daily
 Route of administration: Oral gavage
 Dose volume: 1 ml/kg
 Formulation/Vehicle: PEG400, 1.2 eq. NaOH, 0.3 eq. HCl
 Species/Strain: Marshall Beagle dogs

Number/Sex/Group: 4
Age: 6.5 - 7.5 months
Weight: Mean weight in Males – 7.4-7.7 kg, Females –
6.5-6.6 kg
Satellite groups: None.
Unique study design: None.
Deviation from study protocol: No impact deviations. **Statistical analyses
were not performed for this study.**

Observations and Results

Mortality

No mortality was observed on the study.

Clinical Signs

Dogs were observed daily. Time related observations were recorded at 30 min prior to dosing and at 1, 2, 4, 6, and 24 hours on Days 0, 7, 14, 21, 28, 63, 93, 125, 154 and 177. Clinical signs included excessive salivation at 5, 15 and 45 mg/kg/day in males and females as well as an increase in the frequency of soft, mucoid and pale feces in males and females at 45 mg/kg/day compared with controls. Vehicle treated animals also showed soft (slight-severe), pale and mucoid feces as well as salivation. Slight vomiting was also seen across male dose groups and in females at the low and high dose groups. Females showed slight lacrimation at the low dose.

Body Weights

Body weights were measured on Day -5, Day 0, weekly during dosing and the day prior to necropsy. Males showed a decrease in body weight at 45 mg/kg/day from Week 4 and on. At week 26, high dose males had a 7.6% decrease in body weight compared with controls.

Females showed an increase in body weight from Week 6 and on. At week 26, the body weight increase was up to 5.4% in the mid and high dose groups compared with controls.

Feed Consumption

Food consumption was measured weekly. Males showed a decrease in food consumption at the high dose of up to 5.6% compared with controls. Females showed an increase in food consumption of 3.2% at the low dose, 6.3% at the mid-dose, and 1.3% at the high dose.

The food consumption changes were consistent with the body weight changes in males and females.

Ophthalmoscopy

No test article related ophthalmic abnormalities

ECG

No test article related ECG abnormalities were observed at 1, 3 and 6 months. Second degree atrio-ventricular block was observed in 2 dogs in the vehicle group, 1 dog in the 15 mg/kg group, and 2 dogs at 45 mg/kg. These findings were not considered related to

treatment since they were also observed pre-dose and in vehicle controls. In addition, second degree atrio-ventricular block can occur when animals are excited following a change in vagal tone.

Hematology

Hematology was measured on Day -6, Day 23, Day 57, Day 94, and Day 171 (Week 25). Reticulocytes were decreased in females at all dose groups at Week 14 ranging from 17.7-38.4% and at Week 25 ranging from 21.2%-50.9% compared with pre-dose values. In males, the reticulocyte counts were only slightly decreased when compared to pre-dose values (8.6%) at the high dose.

Coagulation

Coagulation was measured on Day -6, Day 23, Day 94, and Day 171. Coagulation changes at Week 25 included a decrease in APTT (16% vs predose value) in high dose males, as well as increases in fibrinogen in high dose males (14.1% vs. pre-dose), low dose females (27.3% vs. pre-dose) and high dose females (48.8% vs. pre-dose).

Clinical Chemistry

Clinical chemistry was measured on Day -6, Day 23, Day 94, and Day 171 (Week 25). Clinical chemistry changes were mainly seen in high dose animals and included decreases in triglycerides, decreases in cholesterol, increases in total bilirubin (indirect and direct in males at Week 4, 14 and 25 as well as females at Week 25) and changes in ALT, AST and ALP compared with the group mean Week 0 baseline values. The changes are summarized below.

Triglyceride group mean values were reduced in high dose females at 4 weeks (27.5%) and appeared to recover by the end of the study. Mid-dose males showed mean triglyceride decreases at 4 weeks (20.5%), 14 weeks (20.5%) and 25 weeks (25.6%), and high dose males at 4 weeks (27.8%), 14 weeks (30.6%) and 25 weeks (25.0%). Cholesterol group mean changes were similar to triglyceride changes with reductions in high dose females after 4 weeks (24.3%), in mid-dose male at 14 weeks (23.5%) and 25 weeks (26.8%), and in high dose males at 4 weeks (28.4%), 14 weeks (19.9%) and 25 weeks (34.0%).

Increases in group mean total bilirubin, direct bilirubin and indirect bilirubin were seen at various timepoints. In males, total bilirubin increases were greatest at 4 weeks and 14 weeks and appeared to decline by 25 weeks. In females, total bilirubin levels appeared to increase with time from 4 weeks, to 14 weeks (1.8X) and out to 25 weeks (1.9X) compared to pre-dose values. Total bilirubin changes are summarized in Table 10. Direct bilirubin increased at 14 weeks in high dose males (3X compared to pre-dose) and decreased to pre-dose values at 25 weeks (0.01 mg/dl). In high dose females, direct bilirubin was not detected pre-dose (0.0 mg/dl) and showed increases at 14 weeks (0.02 mg/dl) and 25 weeks (0.03 mg/dl). Compared to pre-dose values, indirect bilirubin was increased in mid-dose males at Week 4 (2.3X) and Week 14 (2.0X), in high dose males at Week 4 (1.9X) and Week 14 (2.0X), and in high dose females at Week 4 (1.7X), Week 14 (1.6X), and Week 25 (1.8X).

Table 10 Bilirubin Group Mean Fold-change from Baseline at Weeks 4, 14 and 25

Total Bilirubin	Week 4		Week 14		Week 25	
	Male	Female	Male	Female	Male	Female
Vehicle	1.2	1.2	1.2	1.2	0.8	1.3
5 mg/kg/day	1.1	1.4	1.3	1.3	0.9	1.2
15 mg/kg/day	2.3	1.3	2.0	1.4	1.5	1
45 mg/kg/day	1.8	1.7	2.1	1.8	1.3	1.9

Alanine transaminase (ALT) group mean values were increased in mid-dose and high-dose males and females throughout the dosing period (Table 11). At the mid-dose, the greatest increase in males and females was observed at Week 25 compared with pre-dose values. At the high dose, increases were seen at all timepoints. Male No. 61 showed the highest increase in ALT of 62.3X compared to pre-dose at Week 14. In female No. 163 an increase of 14X over pre-dose was seen at Week 14 and Week 25.

Table 11 ALT Group Mean Fold-change from Baseline at Weeks 4, 14 and 25

ALT	Week 4		Week 14		Week 25	
	Male	Female	Male	Female	Male	Female
Vehicle	1.3	1.1	1.4	1.1	1.3	1.2
5 mg/kg/day	1.3	1.0	1.3	1.0	1.3	1.0
15 mg/kg/day	1.4	1.4	1.5	1.3	1.6	1.7
45 mg/kg/day	4.2	1.9	22	5.3	14	6.4

Aspartate transaminase (AST) group mean values were increased in high dose males at Week 14 (4X); the increase was mainly due to one male (No. 61, the same male with the high ALT increase) AST increase of 15X compared to the predose baseline value in this animal.

Alkaline phosphatase (ALP) was increased in high dose males at Week 4 (1.3X), Week 14 (1.6X), and Week 25 (1.4X) and in one high dose female (2.6X at Week 14) compared with pre-dose values. Male No. 61 exhibited the highest increase in ALP of 2.2X over baseline.

Urinalysis

Urinalysis was evaluated on Day -6, Day 23, Day 94, and Day 171. High dose males showed increased levels of bilirubin at Week 4 and Week 14 and urobilinogen in urine at Week 4 and Week 25 compared to controls and pre-dose levels.

Gross Pathology

One high dose female had a slightly pale liver of all lobes (No. 161) and another high dose female presented with slightly swollen liver lobes (No. 164). These animals had liver histopathology findings that correlated with the gross liver findings as described further below.

Organ Weights

Liver weights were slightly increased (absolute and normalized to body and brain weights) in males across treatment groups and in high dose females compared to controls (Table 12). The greatest increase was seen in high dose females (25% increase in liver weight normalized to brain).

Table 12 Liver Weight Changes in Male and Female Dogs

Liver		Absolute Weights	% Brain	% Body Weight
Vehicle	Sex	% Change Control	% Change Control	% Change Control
5	Male	10	16	8.1
15	Male	8.8	11	5.6
45	Male	15	16	24
45	Female	27	25	22

A decrease in prostate weight was seen in high dose males by absolute prostate weight (17%), prostate normalized to brain (16%), and prostate normalized to body weight (12%).

Adrenal gland mean weights were decreased in high dose females compared to mean control weights (absolute - 20%, %brain, 22%, % body, 24%). Histopathology findings in the adrenal glands included swollen zona fasciculata cells and vacuolization.

Increases in spleen weights were seen in mid and high dose females (Table 13). Higher red blood cells in the red pulp of spleen at mid dose correlated with higher weight in one mid dose female.

Table 13 Spleen Weight Changes in Female Dogs

Spleen		Absolute Weights	% Brain	% Body Weight
Vehicle	Sex	% Change Control	% Change Control	% Change Control
15	Female	52	50	42
45	Female	32	42	24

Histopathology

Adequate Battery

Yes.

Peer Review

Yes.

Histological Findings

Liver

Liver findings were seen at 45 mg/kg day in two males and two females. Minimal-moderate multifocal nonzonal hepatocellular necrosis was seen in male No. 62 (several small foci, minimal), female No. 164 (some small foci, minimal) and male No. 61 (several small and large foci, moderate). These necrotic foci were associated with hemorrhage, neutrophils and correlated with increases in ALT in these animals (No. 62

– 14X ALT, No. 164 - 4.6X ALT , No. 61 - 62X ALT compared with baseline). Male No. 61 also showed minimal periportal mixed inflammatory infiltrates (mainly granulocytes) which may have correlated with increases in ALP (2.2X over baseline). Brown pigmented Kupffer cells and macrophages were seen in the same two high dose males and two high dose females. Perl's stain was used in one male (No. 61) to confirm that the macrophage contents were positive for an iron-pigment indicative of hemosiderin. In addition, one male at the low dose and two high dose females (No. 162 and No. 164) showed mononuclear/phagocytic uptake of necrotic hepatocytes. The liver histopathology findings are summarized in Table 14.

Table 14 Summary of Microscopic Liver Findings in Male and Female Dogs

Diagnosis	Severity	Males (N=4)				Females (N=4)			
		0	5	15	45	0	5	15	45
Brown, pigmented Kupffer cells/macrophages	Grade 1	-	-	-	2	-	-	-	2
Mixed cell infiltrates	Grade 1	-	-	1	1	-	-	-	-
Necrosis	Grade 1	-	-	-	1	-	-	-	1
	Grade 3	-	-	-	1	-	-	-	
MPS*-aggregates with necrotic hepatocytes	Grade 1	-	-	-	-	-	-	-	2
	Grade 2		1						
Extramedullary hematopoiesis	Grade 1	2	1	3	1	-	-	2	2
	Grade 2	-	-	-	-	-	1	-	-

MPS – mononuclear phagocytic system

Small intestine (duodenum, jejunum)

Vacuolization of the apical enterocytes was seen in the duodenum of one high dose male and the jejunum of another high dose male – these findings correlated with an increase in fat droplets. In addition dilatation of the duodenum crypts and lacteals was seen at a higher incidence in treated males over controls.

Gall Bladder

Notable gall bladder findings included brown pigmented macrophages (Grade 1), prominent vacuolated epithelial cells (grade 1) and edema in 1/4 high dose males and 1/4 high dose females. A summary of potential treatment related findings is summarized in Table 15 below.

Table 15 Summary of Microscopic Gall Bladder Findings in Male and Female Dogs

Diagnosis	Severity	Males (N=4)				Females (N=4)			
		0	5	15	45	0	5	15	45
Brown, pigmented macrophages	Grade 1	1	-	-	2	-	-	1	2
Edema	Grade 1	-	-	1	-	-	-	-	1
Infiltration granulocytic	Grade 1	-	1	-	1	-	-	-	-
Prominent vacuolated epithelial cells	Grade 1	2	-	1	2	-	-	1	3

Pancreas

In females an increase in the incidence of mononuclear cell infiltrates of the pancreas was seen in 1/4 mid dose and 2/4 high dose females

Adrenals

In males, the adrenal glands showed swollen zona fasciculata cells (Grade 1) in 1/4 mid dose and 1/4 high dose males. In females vacuolization was seen in 1/4 controls, 2/4 low dose, and 2/4 mid-dose females.

Heart

In the heart, there appeared to be a slight increase in mononuclear cell infiltrates at the high dose (4/4 vs. 1/4 controls); this was not seen in females. Other findings of interest but observed infrequently in only one animal included a hematocyst in one low dose male (No. 23, 1-3mm in size in the tricuspid valve), prominent eosinophilic fibre in one mid dose male and chronic inflammation in one low dose female. Due to the low incidence and inconsistent dose response, the relationship of these findings to treatment is unknown. The heart findings are summarized in Table 16.

Table 16 Summary of Microscopic Heart Findings in Male and Female Dogs

Diagnosis	Severity	Males (N=4)				Females (N=4)			
		0	5	15	45	0	5	15	45
hematocyst	Grade 2	-	1	-	-	-	-	-	-
Infiltration mononuclear cells	Grade 1	1	2	1	4	2	1	1	2
Prominent eosinophilic fibre	Grade 1	-	-	1	-	-	-	-	-
Chronic inflammation	Grade 2	-	-	-	-	-	1	-	-

CNS Findings

CNS findings of unknown relationship to treatment due to the low frequency or lack of a dose response included optic nerve hemorrhage in 1/4 high dose males, spinal cord gliosis (1/4 high dose males) and hemorrhage (cervical 2/4 low-dose and 1/4 mid-

dose males; thoracic 1/4 at low dose and 1/4 at high dose). The sponsor considered the perivascular cuffing and minimal focal gliosis in the brain and the gliosis in the spinal cord to be incidental. In the high dose male with perivascular cuffing, focal mononuclear infiltrates of the meninges was also seen. Focal brain vacuolization occurred in the white matter in one low dose male (not described for the other male), and the medulla of the mid-dose male. Focal brain gliosis occurred in the medulla oblongata in both female No. 162 and 164. The CNS findings are summarized in Table 17.

Table 17 Summary of Microscopic CNS Findings in Male and Female Dogs

Tissue	Diagnosis	Severity	Males (N=4)				Females (N=4)			
			0	5	15	45	0	5	15	45
Optic Nerve	Hemorrhage	Grade 1	-	-	-	1	-	-	-	-
Spinal Cord - cervical	Gliosis	Grade 1	-	-	-	1	-	-	-	-
Spinal Cord - cervical	Hemorrhage	Grade 1	-	2	1	-	1	1	1	-
Spinal Cord - thoracic	Hemorrhage	Grade 1	-	1	-	1	-	-	-	-
Brain	Gliosis	Grade 1	-	-	-	-	-	-	-	2
Brain	Granulocytic infiltrate	Grade 1	-	1	2	-	-	-	1	-
Brain	Mononuclear cell infiltrate	Grade 1	-	-	2	1	-	-	-	2
Brain	Perivascular cuffing	Grade 1	-	-	-	1	-	1	-	-
Brain	Vacuolization	Grade 1	-	2	1	-	-	-	-	-

Epididymides:

Minimal focal vacuolization in 1/4 mid and 1/4 high dose male, presence of cellular debris in 1/4 high dose males and hemorrhage in 1/4 mid dose males.

Testes

Testes findings in males included atrophy/tubular hypoplasia in 1/4 control animals, 2/4 low dose, and 2/4 high dose animals (Grade 1 and Grade 2 severity scores).

Kidneys

An increase in kidney mononuclear cell infiltrates (grade 1) was seen in treated females in 1/4 low dose, 2/4 mid-dose and 3/4 high dose females. In males, there was congestion (grade 1) in 1/4 high dose males, mixed cell infiltrates in 1/4 mid and 1/4 high dose males (Grade 1) and chronic inflammation in 1/4 low dose and 1/4 high dose males.

Spleen

In the spleen, there was a higher incidence of erythrocytes in the red pulp region at a greater severity (Grade 3 and 4 in males and females) than that seen in controls (Table

18). The sponsor considered this finding to be an artifact related to the exsanguination procedure and residual blood.

Table 18 Summary of Microscopic Spleen Findings in Male and Female Dogs

Tissue	Diagnosis	Severity	Males (N=4)				Females (N=4)			
			0	5	15	45	0	5	15	45
Spleen	Erythrocytes red pulp	Grade 1	1	3	2	2	3	3	1	-
		Grade 2	3	-	1	-	1	-	2	2
		Grade 3	-	-	-	1	-	1	-	1
		Grade 4	-	-	-	1	-	-	1	1

Special Evaluation

None.

Toxicokinetics

The toxicokinetics of TMC435 administered by oral gavage to male and female dogs was evaluated on Day 28, Day 78 and Day 177 (177 (C_{max} – 1.7X, AUC – 1.7X) and is summarized in Table 19. Overall, female dogs had greater AUC (up to 75% on Day 177 in high dose females) and C_{max} (up to 77% on Day 177 in high dose females) exposures compared to male dogs. AUC increased with dose greater than dose proportionally. While C_{max} increased with dose greater than dose proportionally at 15 mg/kg, and approximately dose proportionally at 45 mg/kg. Accumulation was seen in high dose female dogs for both AUC and C_{max} from Day 28 to Day 177 (C_{max} – 1.8X, AUC – 2.2X) and from Day 78 to Day 177 (C_{max} – 1.7X, AUC – 1.7X).

Table 19 Summary of Toxicokinetic Parameters in Male and Female Dogs Following Daily Oral Gavage of TMC435 for 6 months

	Male			Female		
	Day 28					
Dose (mg eq./kg/day)	5	15	45	5	15	45
C _{max} (µg/ml)	2.02	20.9	46.5	2.74	24.6	39.6
AUC _{0-24 h} (µg.h/ml)	8.25	110	499	9.62	104	381
	Day 78					
Dose (mg eq./kg/day)	5	15	45	5	15	45
C _{max} (µg/ml)	2.00	13.6	40.7	3.36	14.0	41.9
AUC _{0-24 h} (µg.h/ml)	7.52	70.4	431	12.6	74.1	445
	Day 177					
Dose (mg eq./kg/day)	5	15	45	5	15	45
C _{max} (µg/ml)	2.42	14.3	41.0	2.92	17.2	72.7
AUC _{0-24 h} (µg.h/ml)	8.98	69.9	475	10.4	79.7	833

Dosing Solution Analysis

Dose formulation and stability analyses met the protocol defined acceptance criteria of +/-15%. Test article was not measured in the vehicle formulation.

Study title: TMC435350: Toxicity Study by Oral Gavage Administration to Beagle Dogs for 39 Weeks Followed by a 13 Week Recovery Period

Study no.: **TMC435-TiDP16-NC207/TOX9256**

Study report location: Johnson & Johnson Pharmaceutical
Research and Development

Conducting laboratory and location:

(b) (4)

Date of study initiation: January 28, 2009

GLP compliance: Yes, with the exception of the tissue homogenate analyses.

QA statement: Yes

Drug, lot #, and % purity: TMC435350, ZR494617PFA071, 98.6%

Key Study Findings

- **Mortality** – two females sacrificed moribund one at 15 mg/kg/day (Day 273) and one female dosed at 45 mg/kg/day (Day 18); the sponsor considered these early deaths to be caused by gavage errors however these two females had the highest concentrations of TMC435 in the liver, therefore it is possible that TMC435 contributed to the early deaths.
- **Clinical signs** – salivation, loose or liquid feces, and vomiting.
- Increase in MCHC, lymphocytes, ALT, AST, ALP, bile acids. Decrease in APTT, cholesterol (males only, increases in cholesterol and triglycerides were seen in females).
- **ECGs** – increase in P-wave that correlated with PR-interval prolongation in mid and high-dose males (up to 18 msec in one high dose male at Week 26 - 2 h) and females across dose groups (up to 28 msec in one mid-dose female at Week 26 - 2 h). QTcV prolongation in one low dose female (up to 16 msec at Week 39 - 24 h) and one high dose female (up to 21 msec at Week 26- 2 h).
- **Heart:** mixed cell infiltrates (1/4 males and 1/4 females at the low-dose, 1/4 males at the mid-dose, 1/4 females at the high-dose) and papillary projections of the epicardium (atrium) in 1/4 control males and 2/4 mid-dose males. In early death females, agonal cardiac hemorrhage (1/4 at the mid-dose and 1/4 at the high-dose) indicative of cardiac hypoxia/the moribund condition of the animals was observed.
- **Adrenal:** foamy/vacuolated cells, mononuclear infiltrates, and prominent vacuolization of the zona glomerulosa (grade 1, 2/4 males and 1/4 females at the mid-dose and 1/4 males at the high dose).
- **Pancreas:** Chronic inflammation, mononuclear cell infiltrates and prominent apoptosis (2/4 high dose females, grade 1).

- **Liver:** Subcapsular fibrosis, mixed cell infiltrates, mononuclear phagocyte aggregates with necrotic hepatocyte.
- **Small intestine:** dilatation of lacteals (ileum, duodenum), vacuolation (ileum, jejunum) crypt dilatation (duodenum), congestion (jejunum). Atrophy was seen in one animal.
- **Testes/Epididymides:** Tubular atrophy/hypoplasia of the testes was seen in 2/4 controls, 2/4 low dose, 1/4 mid-dose and 3/4 high dose males.
- **NOAEL** – 5 mg/kg/day based on early death at 15 mg/kg/day (male AUC at 5 mg/kg/day - 4338 ng*h/ml, female AUC at 5 mg/kg/day - 9308 ng*h/ml, mean – 6823 ng*h/ml; 0.1X multiple over clinical AUC).

Methods

Doses:	0, 5, 15, 45 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	1 ml/kg
Formulation/Vehicle:	Polyethylene glycol (PEG400) with 1.2 eq. Sodium hydroxide (NaOH) and 0.3 eq. Hydrochloric acid (HCl).
Species/Strain:	Pure bred beagle dogs (b) (4)
Number/Sex/Group:	4/sex/group main study, 2/sex in control and high dose groups for 13-week recovery
Age:	6-7 months at treatment initiation
Weight:	6.4 to 8.9 kg (males) and 5.0 to 6.9 kg (females).
Satellite groups:	None.
Unique study design:	Measurement of liver and heart TMC435 levels
Deviation from study protocol:	None reported.

Observations and Results

Mortality

Two females were sacrificed moribund prior to the end of the 39 week study. Female No. 936 (45 mg/kg/day) was euthanized on Day 18 immediately following dose administration the cause of death was probably dose inhalation. Clinical signs following dosing included slight cough, gasping, labored breathing, underactivity and salivation. 2 hours after dosing, the animal deteriorated rapidly and was euthanized. Gross necropsy findings included congestion of the lungs and GI tract (colon and duodenum) with copious frothy fluid in the trachea (coming from the lungs). Microscopic findings included agonal congestion and hemorrhage of several tissues. Acute inflammation was seen in the trachea and lungs. Microscopic lung findings of acute, fibrinous bronchopneumonia and congestion/hemorrhages correlated with the gross lung changes.

Female No. 928 (15 mg/kg/day) was euthanized on Day 273 following an apparent seizure; the sponsor thought it was likely that No. 928 also aspirated test article into its lungs since the necropsy findings were similar between female Nos. 928 and 936.

Frothy liquid was present in the trachea and lungs, the lungs showed patchy congestion and were firm from an incomplete collapse. The tonsils were swollen and the stomach contained a clear and frothy fluid, the stomach body had a few congested areas and there was diffuse congestion on the mucosa of the cecum, duodenum and jejunum. Microscopic findings showed congestion in several tissues and moderate lung congestion with intraalveolar pale eosinophilic material, flattened desquamated tracheobronchial epithelium and granulocytic infiltration. The study report notes that the animals' swollen tonsils correlated to lymphoid hyperplasia. Agonal cardiac hemorrhage was seen in both early death females indicative of cardiac hypoxia/the moribund condition of the animals.

The dosing procedure was amended following the death of female No. 936 to using a 3-way tap at the end of the gavage tube. However, despite the change in dosing, 5 more dogs showed respiratory distress (coughing or vomiting immediately or shortly after dosing) across dose groups including one control dog. Three males took a one day dosing holiday to recover.

Clinical Signs

Visual inspections were performed twice daily. Kennels were inspected once daily. Detailed observations were recorded daily during the first two weeks of treatment and Weeks 4-7, twice weekly during Week 3 (middle and end of each week) and Weeks 8-11, weekly during Weeks 12 to 19 and once every two weeks from Week 20. Clinical signs were mainly seen at the high dose of 45 mg/kg/day and included salivation and loose or liquid feces. Vomiting was seen in all groups including controls.

Body Weights

Body weights were measured weekly during the acclimatization period, on the day of dosing (Week 0), weekly during treatment and prior to necropsy. No treatment related effects on body weight or body weight change were observed. A transient decrease in body weight in high dose males occurred at around Week 4 (8%) to Week 19 (4%).

Feed Consumption

Food consumption was measured weekly. No treatment related changes in food consumption were observed.

Ophthalmoscopy

Eye examinations were performed prior to dosing and Week 39 by binocular indirect ophthalmoscope and slit-lamp microscope. No test article related findings were observed by ophthalmoscopy.

ECG

ECGs were evaluated at pre-dose, Week 26 at 2 hours post-dose, Week 26 at 24 hours post-dose, Week 39 at 2 hours post-dose, Week 39 at 24 hours post-dose and Week 13 of recovery. Statistically significant group mean changes included a slight increase in P wave at Week 26 - 24 hours and at Week 39 - 2 hours after dosing in mid (39 vs. 33 - week 26, 39 vs. 32 week 39) and high dose (36 vs. 33 week 26, 36 vs. 32 week 39) males. A slight increase in PR interval was seen in two high dose males at Week 26 at 2 hours and at 4 hours. For the male with the largest PR interval increase of 18 msec at

2 hours at Week 26, the PR interval remained prolonged by 16 msec out to 24 hours at Week 26. In females, statistically significant increases in PR were seen at Week 26-24 hours in low dose (103 vs. 84), mid-dose (93) and high dose (91) females. PR interval was increased in one mid-dose female by 28 msec at Week 26-2 hours and remained prolonged by 14 msec at Week 26 – 24 hours. PR interval trended towards normal values by Week 39 – at 24 hours. A decrease in P wave was seen in high dose females at Week 39-2 hours (31 vs 36).

An increase in QTcV was seen in one low dose female (16 msec or 7% increase from baseline) at Week 39 at 24 hours. Prolonged QTcV was also seen in one high dose female (21 msec or a 9.4% change from baseline) at Week 26 at 2 hours.

Following the 13 week recovery period, there were no ECG abnormalities observed,

Hematology

Hematology was evaluated at baseline, Week 26 and Week 39. MCHC was statistically significantly increased at Week 26 in males (mid-dose 2.4%, high-dose 2.9%) and females (mid-dose not statistically significant – 2.3%, high dose – 1.2%). Lymphocyte counts were increased in females across dose groups at Week 26 (low dose 18%, mid-dose 16%, high dose 25%) and Week 39 (low dose 17%, mid-dose 13%, high dose 9%), although values appeared to reverse at Week 39.

Coagulation

Coagulation was evaluated at baseline, Week 26 and Week 39. Slight decreases in APTT were seen in males and females. In males, APTT decreases in a few animals led to statistically significant decreases on a group mean basis at the low dose (17%), mid-dose (20%) and high dose (24%) groups at Week 26 and remained slightly reduced at Week 39 (up to 15% in the high dose group) compared with the control group. In females, APTT decreases were statistically significantly decreased at both the mid-dose (18%) and high dose (15%) groups at Week 26 and remained slightly reduced at Week 39 (9% at mid-dose, 7% at high dose) compared with controls.

Clinical Chemistry

Clinical chemistry was evaluated at pre-dose, Week 13, Week 26, and Week 39. ALT was statistically significantly increased in high dose males and females at Week 13 and 39 compared to pre-dose baseline values. Male No. 931 at the high dose had the highest increase in ALT at Week 39 of 66-fold over baseline. Male No. 931 also had high AST (5X), total bilirubin (2X) as 100% indirect bilirubin and bile acids (7X vs controls, no baseline value collected for bile acids) at Week 39. Female No. 938 had the highest increase in ALT (7.7X) that correlated with an increase in AST (2X baseline) and GGT 3X baseline). The ALT changes are summarized in Table 20.

Table 20 ALT Group Mean Fold-change from Baseline at 45 mg/kg/day at Weeks 13, 26 and 39

ALT	Week 13		Week 26		Week 39	
	Male	Female	Male	Female	Male	Female
Vehicle	1.3	1.2	1.2	1.1	1.1	1.0
45 mg/kg/day	1.6	2.6	1.7	2.8	10.4	3.6

AST was statistically significantly increased in high dose females (55%) at Week 39. The increase was mainly due to female No. 938 that had the highest ALT increase over baseline (7.7X), with an AST value 2X baseline at Week 39.

There were no statistically significant changes in ALP however group mean increases were seen that were mainly due to two males (No. 937 and 939) that had high values at Week 13 (up to 5X), Week 26 (up to 4X) and at Week 39 (up to 4X) compared to baseline. In females, slight increases of ALP were seen in mid-dose (24%) and high dose (23%) females at Week 13 compared to baseline that were due to one female per group showing increases of ~2X.

Bile acids were increased compared to controls (baseline values were not collected) at Week 39 in males (mid dose 1.3X, high dose 4X) and females (mid dose – 3X, high dose 6X). The increase in the group mean bile acids in females were attributed to female Nos. 938 (5X) and 940 (21X) with the highest increases compared to controls.

Decreases in cholesterol were seen in males and were statistically significant in high dose males at Week 13 (15%) and Week 39 (6%) compared to baseline. In females increases in cholesterol were observed across dose groups including the low dose (up to 75%); increases in triglyceride were also seen at the high dose at Week 13 and 26.

There were no clinical chemistry abnormalities following the recovery period.

Urinalysis

Urinalysis was performed prior to treatment, at Week 26 and Week 39. No test article related changes in urinalysis were observed.

Gross Pathology

The sponsor did not identify TMC435 related gross pathology findings. Low incidence findings that could not clearly be attributed to TMC435 but did appear in the treatment group and not in controls included enlarged popliteal lymph nodes in 1/4 low and 1/4 mid dose females and congested lungs in 1/4 mid-dose and 2/4 high dose females.

Organ Weights

In males, organ weight changes included a decrease in prostate weights in high dose males (19% normalized to body weight, 21% normalized to brain weight) and an increase in thyroid weights (45% normalized to body weight, 43% normalized to brain weight) compared to controls.

In females, organ weight changes included increased ovary weights at the high dose (33% normalized to body weight, 30% normalized to brain weight), decreased spleen weights (23% normalized to body weight, 25% normalized to brain weight), and an increase in thyroid weights (36% normalized to body weight, 33% normalized to brain weight).

Histopathology

Adequate Battery

Yes.

Peer Review

Yes.

Histological Findings

Adrenal gland: Findings in males included foamy/vacuolated cells (grade 1, 1/4 at the low, 1/4 at the mid and 1/4 at the high dose groups, mononuclear infiltrates (grade 1, 1/4 at the low and 1/4 at the mid), and prominent vacuolization of the zona glomerulosa (grade 1, 2/4 at the mid-dose and 1/4 at the high dose). Prominent vacuolization of the zona glomerulosa was also seen in females (grade 1, 1/4 at the mid-dose); other adrenal gland findings in females were similar to the control incidence.

Gall Bladder: In males, gall bladder findings included granulocytic (grade 1, 1/4 at the mid-dose, 1/4 at the high-dose) and mononuclear infiltrates (grade 2, 1/4 at the low-dose) as well as epithelium vacuolization (grade 1, in 1/4 at the low-dose, 1/4 at the mid-dose, and 3/4 at the high-dose).

Heart: in males, findings that were seen at a greater incidence than controls included mixed cell infiltrates (grade 1, 1/4 at the low-dose, 1/4 at the mid-dose) and papillary projections of the epicardium (atrium) in 1/4 controls and 2/4 mid-dose males. Heart findings in females also included mixed cell infiltrates (grade 1 in 1/4 at the low-dose and 1/4 at the high-dose). **Agonal cardiac hemorrhage was seen in the early death females in 1/4 at the mid-dose and 1/4 at the high-dose indicative of cardiac hypoxia/the moribund condition of the animals.**

Kidneys: in males, kidney findings included tubule basophilia (grade 1 in 2/4 at the low-dose and 2/4 at the mid-dose), hyaline casts ((grade 1 in 2/4 at the low-dose, 1/4 at the mid-dose and 2/4 at the high-dose), transitional cell hyperplasia (grade 1 in 1/4 at the low-dose and 1/4 at the high-dose), and chronic inflammation (grade 1 in 1/4 at the low-dose and grade 2 in 1/4 at the mid-dose). In females, congestion of the kidneys was seen in 1/4 at the mid-dose (grade 1) and 1/4 at the high-dose (grade 2).

Lacrimal glands: in females, lacrimal glands showed granulocytic infiltrates (2/4 at the mid-dose and 1/4 at the high-dose) and mononuclear infiltrates (1/4 at the mid-dose, grade 2, and 1/4 at the high-dose, grade 1)

Liver: in males, findings were seen at a low incidence making it difficult to interpret the data. The listed findings were only seen in 1 dog/group however, the findings are potentially adverse so are included. Bile duct proliferation was observed in 1/4 high

dose males (grade 2, No 933). This animal had the highest measured liver concentrations that were 2.2X higher than the group mean. Liver findings associated with the bile duct proliferation included small hepatocytes, fibrosis, chronic inflammation, brown pigmented macrophages, mixed cell infiltrates, centrilobular glycogen content (Grade 4); although this was only seen in one animal, it was a notable finding and may be test article related since it was seen at the high dose. No. 933 appeared to have signs of inflammation and vacuolization of multiple organs (see further description below). Subcapsular fibrosis (grade 1) was seen in 1/4 low dose males, 2/4 mid dose females, as well as mixed cell infiltrates (grade 1) in 1/4 high dose males and 1/4 low dose females. MPS aggregates with necrotic hepatocytes (grade 1) was seen in 1/4 low dose females and 2/4 mid dose females.

Lung:

In females, lung findings included congestion (1/4 controls, 2/4 mid-dose; grade 1 and grade 3, and 3/4 high dose; two grade 1 and 1 grade 3), flattened desquamated epithelium of the bronchi (1/4 mid dose and 1/4 high dose females, grade 2), foamy macrophages (1/4 low-dose and 1/4 mid-dose females, grade 1), hemorrhage (1/4 high dose females, grade 2), granulocytic (2/4 low dose and 1/4 mid-dose females, grade 1) and mixed cell infiltrates (1/4 low dose, grade 1), acute inflammation (1/4 high dose females, grade 2) and chronic inflammation (1/4 low dose females, grade 1).

Lymph nodes: Erythrophagocytosis was seen at a low incidence in lymph nodes in the mesenteric and popliteal lymph nodes in males and females across dose groups. Other findings in the lymph nodes included congestion and granulocytic infiltrates and are summarized in Table 21.

Table 21 Summary of Microscopic Lymph Node Findings in Male and Female Dogs

Tissue	Diagnosis	Severity	Males (N=4)				Females (N=4)			
			0	5	15	45	0	5	15	45
Mesenteric Lymph node	Erythrophagocytosis	Grade 1	-	3	1	1	1	-	-	2
	Popliteal Lymph Node	Erythrophagocytosis	Grade 1	-	-	1	-	-	-	1
	Granulocytic infiltrate	Grade 1	-	-	-	-	-	1	-	-
	Granulocytic infiltrate	Grade 2	-	-	1	-	-	1	-	1

Mammary glands: mammary gland dilatation in treated females was seen in 1/4 low dose (grade 2), 2/4 mid-dose (grade 1 and grade 3) and 3/4 high dose (1 grade 1 severity and 2 grade 2 severity scores) females

Pancreas: Chronic inflammation (males - 1/4 low-dose, grade 1, 1/4 mid-dose, grade 1, 2/4 high dose, grade 1 and grade 2, female – 1/4 low dose, grade 1), mononuclear cell

infiltrates (1/4 low dose males, grade 2) and prominent apoptosis (2/4 high dose females, grade 1) were seen.

Pituitary gland: Generally observed as a background finding, in males pituitary cyst appeared only in treated males in 3/4 low dose (2 grade 1, 1 grade 2), 1/4 mid dose (grade 1) and 2/4 high dose (1 grade 1 and 1 grade 2) males.

Prostate: In males, acute inflammation (1/4 high dose males, grade 2) and chronic inflammation (2/4 mid dose, severity grade 1 and 2, and 3/4 high dose, 2 dogs with grade 1 severity, and 1 dog with grade 2 severity) of the prostate was observed.

Salivary Gland: In females, the mandibular salivary gland showed an increased incidence of mononuclear cell infiltrates (1/4 control, 1/4 low dose, 1/4 mid-dose and 3/4 high dose) and chronic inflammation in 1/4 low dose females. In the parotid gland, atrophy was seen in 1/4 controls and 2/4 mid-dose as well as mononuclear cell infiltrates in 1/4 controls (grade 1), 1/4 low dose (grade 2), 3/4 mid-dose (1 dog with grade 1 severity and 2 dogs with grade 2 severity) and 3/4 high dose (2 with grade 1 severity and 1 with grade 2 severity) females.

Small intestine: Small intestine findings were seen in males in the ileum (dilatation of lacteals in 1/4 mid dose and 1/4 high dose animals, grade 1 and vacuolization in 1/4 mid dose and 1/4 high dose animals, grade 1), jejunum (vacuolization in 2/4 high dose males, grade 2) and duodenum (crypt dilatation, 2/4 control, 3/4 low dose, 1/4 mid-dose, grade 1). In females, small intestine findings were seen in the jejunum (congestion, 1/4 mid dose and 1/4 high dose animals, grade 1) and duodenum (dilatation of the crypts, grade 1, 2/4 low dose, 1/4 mid-dose, and lacteals, grade 1, 1/4 mid-dose, 1/4 high dose) **accompanied at a low incidence by atrophy (1/4 high dose)**, congestion (1/4 high dose) and granulocytic infiltrates (1/4 mid dose)).

Vacuolization of the small intestine was localized to the apical villous epithelium in a previous study using Oil red O stain to show that the vacuoles corresponded with an increase in fat droplets. The sponsor notes that the observed dilated lacteals in the duodenum or ileum of 1/4 males and 1/4 females at the low dose and 1/4 high dose females may be related to the enterocyte vacuolization based on the physiology of fat absorption – after the absorption of fat in the enterocytes at the villous tips, the chylomicrons are passed on to the lacteals (the lymphatics of the intestine).

Testes/Epididymides: Tubular atrophy/hypoplasia of the testes was seen in 2/4 controls (grade 1), 2/4 low dose (grade 1), 1/4 mid-dose (grade 1) and 3/4 high dose males (1 grade 1 and 2 grade 2). In addition, one mid-dose male presented a unilateral small sperm granuloma.

Thymus: in females, prominent cystic/epithelial structures (grade 1) were observed in 1/4 low dose, 2/4 mid-dose, and 1/4 high dose females.

Trachea: in females, trachea findings included congestion in 1/4 at high dose (grade 2), flattened/desquamated epithelium in 1/4 mid-dose and 1/4 high dose (grade 2),

granulocytic infiltrates in 1/4 low dose (grade 1), 3/4 mid dose (grade 1), and 2/4 high dose (grade 1 and grade 2), mononuclear cell infiltrates in 1/4 high dose (grade 1).

Notable individual animal findings:

Male No. 933, the male with high liver levels and bile duct proliferation, also showed signs of inflammation and vacuolization of multiple tissues. Inflammation or inflammatory cell infiltrates (described as infiltration of mononuclear cells or granulocytic infiltration) was seen in the epididymides, gall bladder, liver, lung, pancreas, prostate, salivary gland, and tongue. Vacuolization was seen in the adrenal glands, gall bladder, ileum, jejunum.

RECOVERY

Following a 13 week treatment free recovery period in 2/sex from the vehicle group and 2/sex from the high dose group, findings were seen in the epididymides (vasculitis/perivasculitis, adventitia, bilateral, grade 2 in 1/2 males), and the duodenum (crypt dilatation in 1/2 males and 1/2 females). Findings in the testes, of tubular atrophy/hypoplasia, focal, unilateral were seen in 1/2 controls and 1/2 treated males but the severity was greater in the control animal (grade 2 vs. grade 1), therefore the sponsor did not consider the testicular finding to be related to treatment. However, the vasculitis/perivasculitis of the epididymides observed in the treated male may be associated with the testicular tubular atrophy, therefore these findings may be important since a more severe pathology was associated with the tubular atrophy.

Special Evaluation

TMC435 liver and heart concentrations were evaluated at all dose levels. TMC435 increased with dose greater than dose proportionally in both the liver and heart. The data were highly variable. No clear sex differences were observed. Compared with plasma, liver levels were much higher with liver:plasma ratios ranging from 29-78X in males across dose groups and 3-96X in females across dose groups. Compared with plasma, heart levels were similar to slightly higher than plasma levels with heart:plasma ratios ranging from 0.9-3.7X in males across dose groups and 0.5-1.4X in females across dose groups.

The two female early deaths No. 928 (collapsed on Day 273) and No. 936 (sacrificed on Day 18) had the highest measured liver levels of drug compared to other treated animals (31.6X compared to the average of the other 3 females in the mid dose group). No. 928 also had extremely high heart (327X the mean heart concentration at the mid-dose excluding No. 928) and plasma levels (23.2 ug/mL or 395X the 3 other females in the same group and 46X higher than the high dose group mean plasma concentration) on the day of necropsy and the lowest liver:plasma ratio of 3 because of the high plasma levels. No. 936 showed extremely high liver levels (6.2X the high dose group mean excluding No. 936); plasma and heart concentrations were not collected for this female. Hemodynamic alterations (blood pressure, blood volume) or gavage error may have played a role in the high plasma and tissue concentrations. However, it is also possible that high drug levels directly contributed to the deaths of these two animals. Another possibility is that these dogs may have been mis-dosed with a higher dose level resulting in greater plasma and tissue concentrations.

Toxicokinetics

The TK of TMC435 on Day 90 and Day 273 is summarized in the sponsor's table below (Table 22). C_{max} and AUC exposures increased with dose greater than dose proportionally with the exception of the high dose C_{max} which increased approximately dose proportionally. Overall, females had higher exposures than males with the exception of the high dose AUC and C_{max} on Day 273. Drug accumulation was not seen between Day 90 and Day 273. In high dose females, AUC exposure declined by 55% between Day 90 and Day 273. The calculated TK parameters were highly variable.

Table 22 TMC435 Toxicokinetics Following Daily Oral Dosing for 39 weeks

Dose (mg eq./kg/day)	Male			Female		
	5	15	45	5	15	45
Group	L	M	H	L	M	H
Day 90						
C _{max} (ng/ml)	1138	11793	31642	1648	15848	34840
T _{max} (h)	2	2	2-4	2	2	2-4
AUC _{0-24h} (ng.h/ml)	3839	41538	272715	6181	57838	339207
Day 273						
C _{max} (ng/ml)	1259	10517	36062	2252	17167	24908
T _{max} (h)	2	2	2-8	2	2	2-4
AUC _{0-24h} (ng.h/ml)	4338	47375	297001	9308	70104	151534

Dosing Solution Analysis

Dose formulation analyses met the protocol defined acceptance criteria of +/-15%. Test article was not measured in the vehicle formulation. Stability analyses failed at Week 1 (12-14% less than initial concentration and upon reanalysis, 13-17% lower) and failed to meet the ≤ 10% acceptance criteria. Stability analyses at Week 13 met the acceptance criteria. Therefore, the sponsor did not consider the Week 1 stability results that failed to impact the overall study.

7 GENETIC TOXICOLOGY

Genotoxicity was not observed in TMC435 *in vitro* and *in vivo* studies. Genetic toxicity studies were previously reviewed by Christopher Ellis, PhD.

TMC435350 is not mutagenic, as determined in the Ames test (NC148 & NC173) and the mammalian forward mutation assay in mouse lymphoma cells (L5178Y) (NC130), or clastogenic or aneuploidic as determined in the *in vivo* mammalian micronucleus test in mice (NC172). All studies were performed properly using validated methods and appropriate controls, and were GLP compliant.

8 CARCINOGENICITY

Carcinogenicity studies were not performed because the proposed indication is 12 weeks.

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Fertility

TMC435 related findings in the rat fertility study included an increase in post-implantation loss at the low dose of 50 mg/kg/day and the high dose of 500 mg/kg/day (2.1X compared to controls). Three males showed 100% static sperm: 2/24 (8.3%) male rats at 50 mg/kg/day and 1/24 (4.2%) male rats at 500 mg/kg/day at an incidence higher than historical controls (2008-2012) 3/295(1%). The static sperm finding was associated with small testes and epididymides (unilateral and bilateral) and had a negative impact on pregnancy outcome in 2/3 of the female mating partners. The sponsor argued that the static sperm finding was incidental since it has been seen in control animals previously; however, the incidence of the finding in this study is 4-8X higher than the historical incidence.

Teratogenicity

Teratogenic findings of exencephaly and protruding tongue were observed in the pilot mouse embryofetal study in 6/50 fetuses at 1000 mg/kg (from 2 out of 4 litters) and 4/64 fetuses at 2000 mg/kg (from 2 out of 6 litters). The sponsor provided historical data from the laboratory conducting the study (Janssen R&D Test Facility, Beerse site) that indicated the finding is rare: There were no cases of exencephaly or protruding tongue out of a total of 815 fetuses evaluated (total of 6 mouse embryofetal studies were conducted between 2007-2013). In the pivotal mouse embryofetal study, the dose volume was reduced from 10 ml/kg to 5 ml/kg and the high dose was set at 1000 mg/kg; the teratogenic findings were not reproduced in the pivotal mouse study. The laboratory conducting the pivotal study, (b) (4), provided historical data from a greater number of studies that are in agreement with the Janssen Test Facility site: exencephaly was observed in 2/1950 (0.1%) fetuses (total of 10 studies from 2003-2011). The sponsor noted the following differences that may explain why the teratogenic findings were not reproduced: 1) the mice were from different sources, 2) the testing conditions were different. TMC435 related findings in the pivotal mouse included two maternal deaths at 1000 mg/kg, high post-implantation loss, decreased fetal weight and an increase in skeletal variations. Reproductive toxicity was not observed in the rat embryofetal toxicity study.

Peri- post-natal

In the rat peri- post-natal study, there were two early maternal rat deaths at the high dose of 1000 mg/kg/day on Day 18 (after 12 doses) and Day 10 (after 4 doses). The post-implantation survival index was decreased in TMC435 treated maternal rats at the mid-dose (94%, N=23) and high-dose (93%, N=22) compared with controls (96.2%, N=22). Significant body weight and body weight gain decreases were seen in maternal animals through lactation (up to 40%). In the F1 offspring, small build was seen in many of the fetuses as well as significantly decreased body weight (~20% in offspring from high dose) and body weight gain (up to ~40% in offspring from high dose). A dose dependent increase in kinked tail was observed in 4/24 mid-dose F1 males (from 3 different litters), 2/24 high dose F1 males (two different litters), 1/24 mid-dose F1 females, and 1/24 high dose F1 females. Although treatment related and rare (observed in 2/10 studies conducted between 2008-2011 in 1-2 rats (sponsor did not provide denominator), the kinked tail finding is not considered to be teratogenic, since it was

observed in isolation. In addition, the finding did not impact survival, behavior or mating or future reproductive performance. Offspring from the mid dose of 500 mg/kg and the high dose of 1000 mg/kg/day showed delayed righting reflex and delayed sexual maturation in the females which may be secondary effects related to the observed maternal toxicity. Finally, decreased motor activity (rearing and ambulatory) in the F1 from the high dose indicated physical development effects of treatment with TMC435.

Potential TMC435 related testicular and epididymal toxicity was observed in rats and dogs in the repeat-dose toxicity studies. These findings are noted because static sperm were seen in the rat fertility study. Due to the low incidence across studies and lack of a clear dose response it is not known if the testicular/epididymal findings are incidental or test article related. However, it is compelling that testicular findings were seen in both the rat and dog across studies. These findings are summarized in Table 23 below.

Table 23 Summary of Testicular and Epididymal Findings Across Studies

Study	Study No	Testicular/Epididymal Finding	Control	Low	Mid	High
3-month interim rat	NC179	Testes – Gross – small size moderate, bilateral Diffuse, bilateral atrophy (Grade 4)	0/10	1/1*	0/1*	0/10
6-month rat	NC179	Testes -multifocal atrophy, unilateral (Grade 2)	0/20	--	0/2*	1/20
		Testes -mixed cell infiltrate	0/20	--	0/2*	1/20
1-month dog	NC175	Epididymides – reduced number of spermatozoa, unilateral, moderate	0/3	0/3	0/3	1/3
		Testes – vacuolation in seminiferous epithelium (slight at low dose, moderate at high dose)	0/3	1/3	0/3	1/3
6-month dog	NC226	Testes – Atrophy/tubular hypoplasia (High dose - grade 1 and grade 2 severity)	1/4	2/4	0/4	2/4
		Epididymides – Cellular debris	0/4	0/4	0/4	1/4
		Hemorrhage	0/4	0/4	1/4	0/4
		Vacuolization	0/4	0/4	1/4	1/4
9-month dog	NC207	Testes- tubular atrophy/hypoplasia	2/4	2/4	1/4	3/4
		RECOVERY FINDINGS: Testes – tubular atrophy/hypoplasia, greater severity in control (grade 2) vs. treated (grade 1)	1/2			1/2
		Epididymides – vasculitis/perivasculitis, adventitia, bilateral, grade 2	0/2			1/2

*Note - Only rats with gross findings were evaluated by histopathology in the low (1 rat evaluated) and mid dose (2 rats evaluated) groups in the 3-month interim and 6-month rat toxicity studies.

9.1 FERTILITY AND EARLY EMBRYONIC DEVELOPMENT

Study title: Fertility study of oral TMC435350 in the male and female rat.

Study no.: TMC435-NC190/TOX8714

Study report location: Tibotec Pharmaceuticals Ltd,
Eastgate Village, Eastgate,
Little Island, County Cork, Ireland

Conducting laboratory and location: Global Preclinical Development, Beerse site
Turnhoutseweg 30 B-2340 Beerse, Belgium

Date of study initiation: 29-Jan-2008

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: TMC435350, Batch ZR494617PFA061, purity 96.7%
and Batch ZR494617PFA051, purity 95.9%

Key Study Findings

- Post-implantation loss increase at 50 mg/kg/day (1.7X) and 500 mg/kg/day (2.1X).
- Decrease in body weight gain (11%) in females during gestation.
- Three males showed 100% static sperm: 2/24 male rats at 50 mg/kg/day and 1/24 male rats at 500 mg/kg/day; incidence was higher than historical controls (2008-2012) 3/295 (1%). Associated with small testes and epididymides (unilateral and bilateral) and negative impact on pregnancy outcome in 2/3 female mating partners.
- No NOAEL based on post-implantation loss at 50 mg/kg/day.

Methods

Doses: 0, 50, 150, 500 mg/kg/day

Frequency of dosing: Daily (see details under Study Design below)

Dose volume: 5 mL/kg

Route of administration: Oral gavage

Formulation/Vehicle: Polyethylene glycol 400 (PEG400), 1.2 eq. NaOH, 0.3 eq. HCl

Species/Strain: SPF Sprague-Dawley (CrI: CD®) rats

Number/Sex/Group: 24

Satellite groups: None

Study design: **Males** – dosed daily during 4-week pre-pairing period, throughout pairing period until termination, approximately one week after the fertility rate was confirmed.
Females – dosed daily during a 2-week pre-pairing period, throughout the pairing period and until Day 7 post-coitum and necropsied on Day 14 post-coitum.

Deviation from study protocol: Minor deviations were noted; the deviations had no impact on the validity or integrity of the study.

Observations and Results

Mortality

Three early deaths male No. 201 in the vehicle group was sacrificed at Week 8, female No. 104 in the 500 mg eq/day group was sacrificed at Week 4, and male No. 284 in the 150 mg eq/day group was found dead on Day 11) were determined to be related to dose administration and unrelated to treatment based on gross necropsy findings of perforated esophagus and/or larynx (Nos 201 and 104) as well as lung congestion (No. 284).

Clinical Signs

Clinical signs were observed at the high dose and included audible respiration in 6/24 males, mainly during weeks 5-8, as well as salivation in 14/24 males.

Body Weight

Adverse body weight changes were not seen in males. In females, a slight increase in body weight was seen in the pre-pairing period at the 150 and 500 mg/kg/day dose groups. During pregnancy, a decrease (11%) in body weight gain was seen at the 500 mg/kg/day dose during gestation days 8-13.

Feed Consumption

There was no test article related effect on food consumption in males or females.

Toxicokinetics

Toxicokinetics not evaluated.

Dosing Solution Analysis

The dose solution analyses met the predefined acceptance criteria.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

An increase in post-implantation loss was observed at the low dose (1.7-fold) and the high dose (2.1-fold). There was no effect on the oestrus cycle, copulation index or pre-coital interval. All females mated within 7 days and copulation plugs were confirmed for all pairings.

Fertility/Mating Performance

The sponsor did not observe an effect on fertility or mating performance across the dose groups. The pregnancy outcome was 24/24 in the vehicle group, 23/24 at the low dose (95.8%), 24/24 at the mid-dose, and 22/24 (91.7%) at the high-dose. The one negative pregnancy outcome at the low dose and one of the negative pregnancy outcomes at the high dose were due to insufficient sperm and 100% static sperm in the male mating partners. The second negative pregnancy outcome at the high dose was due to one high dose female sacrificed moribund at Week 4 (female No. 104).

Sperm Analysis*Sperm motility*

A total of 3 treated males showed 100% static sperm – 2/24 male rats at the low-dose (No's 248 and 253) and 1/24 male rats at the high-dose (No. 299). This finding was

associated with small testes and epididymides (unilateral and bilateral) and had a negative impact on pregnancy outcome in 2/3 of the female mating partners.

9.2 EMBRYONIC FETAL DEVELOPMENT

The TMC435 embryofetal studies in the mouse and rat as well as the pilot embryofetal studies were previously reviewed by Christopher Ellis, PhD. Teratogenic findings of exencephaly and protruding tongue were observed in the pilot mouse embryofetal study in 6/50 fetuses at 1000 mg/kg (from 2 out of 4 litters) and 4/64 fetuses at 2000 mg/kg (from 2 out of 6 litters). The sponsor provided historical data from the laboratory conducting the study (Janssen R&D Test Facility, Beerse site) that indicated the finding is rare there were no cases of exencephaly or protruding tongue out of a total of 815 fetuses evaluated (total of 6 mouse embryofetal studies were conducted between 2007-2013). In the pivotal mouse embryofetal study, the dose volume was reduced from 10 ml/kg to 5 ml/kg and the high dose was set at 1000 mg/kg; the teratogenic findings were not reproduced in the pivotal mouse study at a higher N-value per group (N=24 litters/group). The laboratory conducting the pivotal study, (b) (4), provided historical data from a greater number of studies that are in agreement with the Janssen Test Facility site: exencephaly was observed in 2/1950 (0.1%) fetuses (total of 10 studies from 2003-2011). The sponsor noted the following differences that may explain why the teratogenic findings were not reproduced: 1) the mice were from different sources and, 2) the testing conditions were different.

TMC435 related findings in the pivotal mouse included two maternal deaths at 1000 mg/kg, high post-implantation loss, decreased fetal weight and an increase in skeletal variations. Reproductive toxicity was not observed in the rat embryofetal toxicity study.

The embryofetal reviews by Christopher Ellis, PhD are summarized and included below:

Key findings:

- **Pilot studies:** Oral dose range-finding embryo-fetal developmental toxicity studies in Swiss CD-1 mice (0, 150, 500, 1000, 2000 mg/kg) (NC187) and rats (0, 50, 150, 500, 1000 mg/kg) (NC185) were performed and submitted with the original IND submission. In rat, no maternal toxicity was observed and all pregnancy and embryo-fetal parameters (visceral and skeletal observations not made) were unaffected by treatment. No maternal toxicity was observed in the mouse studies, although the cause of death of 2 dams (1000 mg/kg group) was unclear. All pregnancy and embryo-fetal parameters (visceral and skeletal observations not made) were unaffected by treatment except for reduced fetal weight (2000 mg/kg group) and an increased incidence of external malformations, exencephaly (6/50) & protruding tongue (4/64) (both findings observed in 2 different litters), in the 1000 & 2000 mg/kg groups respectively.
- **Pivotal studies:** The NOAEL is 500 mg/kg/day in the rat because no adverse effects observed in high dose dams and offspring. The sponsor justifies the mouse as the second species due to insufficient systemic exposure in rabbit oral administration experiments (NC208 & NC209). In the mouse, the NOAEL is 500 mg/kg/day because adverse effects observed in high dose dams, including

death, and offspring, including lower fetal weight and slightly higher incidences of skeletal variations (incomplete ossification of various bones and full supernumerary 14th ribs).

Study title:	<u>Oral Developmental Toxicity Study of TMC435350 in the Rat</u>
Study #:	NC188
CTD location:	4.2.3.5.2
Conducting laboratory and location:	Johnson and Johnson Pharmaceutical Research & Development, Global Preclinical Development Beerse site
Date of study initiation:	January 8, 2008
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	TMC435350, ZR494617PFA031 and 94.9%

Key findings: The NOAEL is 500 mg/kg/day because no adverse effects observed in high dose dams and offspring.

Methods:

Doses:	0, 50, 150, 500 mg/kg/day (6 through 17d)
Species/strain:	Rat/SPF Sprague-Dawley
#/sex/group (main study):	24
Route/formulation/volume/infusion rate:	oral gavage/PEG400/5 ml/kg/day/NA
#/sex/group (satellite for TK or recovery):	3 (TK)
Age:	12 to 13 wks
Weight:	228 to 287g
Sampling times:	0/1 & 17d (TK)

Results:

In dams:

Mortality (1x/day): None

Clinical signs (1x/day): No test-article related changes noted.

Body weights (2x/wk): No test-article related changes noted.

Food consumption: Remaining food weighed at 0, 6, 10, 14, 18 & 21d. ↓7% between 6 & 9d only (HD).

Gross pathology: No test-article related changes noted.

Gravid uterine weight: No test-article related changes noted.

Toxicokinetics: Fetal plasma concentrations at 21d: 157 (LD), 200 (MD), 549 (HD) ng/ml.

Table 24 Toxicokinetics in the Pregnant Rat on Gestation Day 17

	Day 17		
Dose (mg eq./kg/day)	50	150	500
C _{max} (ng/ml)	2243	3445	3587
AUC _{0-24 h} (ng.h/ml)	16378	28279	29311

Table from sponsor

C-section:

Pregnancy status: 22 (control), 21 (LD), 23 (MD) & 24 (HD) of 24 animals pregnant.

Corpora lutea: No test-article related changes noted.

Implantation sites: No test-article related changes noted.

Implantation loss: No test-article related changes noted.

Resorptions (early and late): No test-article related changes noted.

In offspring:

Viability: No test-article related changes noted.

Gender: No test-article related changes noted.

Body weights: No test-article related changes noted.

External variations: No test-article related changes noted.

External malformations: No test-article related changes noted.

Visceral variations: No test-article related changes noted.

Visceral malformations: No test-article related changes noted.

Skeletal variations:

- ↑ incidence of unossified ventral tubercle of the cervical vertebrae [fetuses: 16.6% (HD) versus 5.8% in control; litters: 54.2 (HD), 47.8 (MD) & 47.6 (LD)% versus 22.7% in control]. Observed in absence of ↓ ossification of metacarpal & sternum so indicative likely of non-adverse transient growth retardation.

Skeletal malformations: No test-article related changes noted.

Histopathology: Only tissues with gross pathology finding examined and if deemed informative potentially. [Adequate Battery: yes (X), no ()]; Peer review: yes (), no (X)]
No test-article related changes noted.

Table 25 Rat Litter Data Following TMC435 Exposure During Organogenesis

Treatment unit: mg eq./kg	Vehicle 0	Low 50	Medium 150	High 500
LITTER DATA				
Number of corpora lutea of pregnancy/pregnant animal (3)	14.9	15.0	15.3	15.5
Number of implantations/pregnant animal (3)	14.1	13.1	12.8	14.6
Pre-implantation loss (%) (3)	5.62	12.65	15.26	5.59
Number of early resorptions / pregnant animal (3)	0.86	0.57	0.87	0.54
Number of late resorptions / pregnant animal (3)	0.00	0.10	0.04	0.00
Total number of resorptions / pregnant animal (3)	0.86	0.67	0.91	0.54
Post-implantation loss (%) (3)	5.90	5.74	6.81	3.73
Number of live fetuses/pregnant animal (3)	13.3	12.5	11.9	14.0
Number of dead fetuses/pregnant animal (3)	0.00	0.00	0.00	0.00
Weight of live fetuses (g) (3)	5.6	5.6	5.5	5.6
Sex ratio (% male fetuses) (3)	51.0	53.2	45.5	45.0
Incidence of malformed fetuses (1)	1/292	1/262	2/273	2/337

Study title:	<u>Embryo-Fetal Toxicity Study by Oral Gavage Administration to CD-1 Mice</u>
Study #:	NC189
CTD location:	4.2.3.5.2
Conducting laboratory and location:	Johnson and Johnson Pharmaceutical Research & Development, Global Preclinical Development Beerse site
Date of study initiation:	January 28, 2008
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	TMC435350, ZR494617PFA031, 94.9%

Key findings: The NOAEL is 500 mg/kg/day because adverse effects observed in high dose dams, including death, and offspring, including lower fetal weight and slightly higher incidences of skeletal variations (incomplete ossification of various bones and full supernumerary 14th ribs). However, no incidences of external malformation (exencephaly or protruding tongue) were observed in this study.

Methods:

Doses:	0, 150, 500, 1000 mg/kg/day (6 through 15d)
Species/strain:	Mouse/CD-1 TM (ICR) BR
#/sex/group (main study):	24
Route/formulation/volume/infusion rate:	oral gavage/PEG400/5 ml/kg/day/NA
#/sex/group (satellite for TK or recovery):	None

Age:	10 to 11 wks
Weight:	23.7 to 34.2 g
Sampling times:	1, 3, 8, 24h on 6 & 15d (TK) (measured in 3/group/time point-no animal bled 2x)

Results:**In dams:**

Mortality (2x/day): 2/24 (HD) at 10d associated with declines in clinical condition (noisy/irregular respiration, underactive, hunched posture, piloerection & ↓ body temp), body weight [↓~6.7 g (21%) versus ↑2.2 g in other HD animals between 8 & 10d] and food consumption. Both animals had total litter resorptions (early). Additionally, one animal had dark red discharge in urino-genital region and slight gas distension of duodenum, jejunum & ileum.

Clinical signs (2x/day): No test-article related changes noted.

Body weights (~1x/day): No test-article related changes noted.

Food consumption (1x/~3days): No test-article related changes noted.

Gross pathology: No test-article related changes noted.

Toxicokinetics: Fetal exposure not determined.

Table 26 Maternal Toxicokinetics in Mice

	150 mg eq./kg/day		500 mg eq./kg/day		1000 mg eq./kg/day	
	Day 6	Day 15	Day 6	Day 15	Day 6	Day 15
C _{max} (µg/ml)	50.6	13.1	57.7	17.5	45.0	25.0
AUC ¹⁾ (µg.h/ml)	404	90.7	458	221	953 ²⁾ [686]	352

¹⁾ AUC_{0-inf} (Day 6) or AUC_{0-24h} (Day 15)

²⁾ AUC_{0-inf} was calculated using 28% extrapolation. AUC_{0-24h} was mentioned between brackets.

Table from sponsor**C-section:**

Pregnancy status: 22 (control), 19 (LD), 23 (MD) & 20 (HD) of 24 animals pregnant.

Corpora lutea: No test-article related changes noted.

Implantation sites: No test-article related changes noted.

Implantation loss: ↑ post-implantation loss (HD)

Resorptions (early and late): ↑ in late resorptions (HD)

In offspring:

Viability: ↑ late embryo-fetal death (HD)

Gender: No test-article related changes noted.

Body weights: ↓ mean fetal and litter weight (HD)

External variations: No test-article related changes noted.

External malformations: No test-article related changes noted.

Visceral variations: No test-article related changes noted.

Visceral malformations: No test-article related changes noted.

Skeletal variations:

- ↑ incidence of fetuses with full supernumerary 14th rib [25.2% (LD), 41.0% (MD) & 52.7% (HD) versus 10.2% (control)] that exceeded the historical control range at the MD & HD.
- Coastal cartilage (8th connected to sternum) in 12/112 fetuses from 4/20 litters (HD) versus 1/127 fetuses from 1/22 litters (control). Uncommon in historical controls but probably of minimal toxicological significance.
- ↑ incidence (MD & HD versus control fetuses) of incomplete ossification of cranial centres (18 & 21% versus 9%), hyoid (6/144 & 8/112 versus 1/127), thoracic vertebrae (11 & 21% versus 2%), metacarpals (17 & 24% versus 11%) & metatarsals (30 & 38% versus 14%). Only the thoracic vertebrae HD value exceeded the historical control range.

Skeletal malformations: No test-article related changes noted.

Histopathology: Only tissues with gross pathology finding examined and if deemed informative potentially. [Adequate Battery: yes (X), no () ; Peer review: yes (), no (X)]
 No test-article related changes noted.

Table 27 Summary of Mouse Embryofetal Litter Data

Group /Sex	Corpora Lutea	Implantations	Resorptions			Live Young			Sex ratio (%M)	Implantation Loss (%)	
Statistical test:	Wi	Sh	Wc	Wc	Total	Male	Female	Total	Wa	Wa	Wa
1F	Mean 13.1 SD 3.88 N 22	12.8 3.74 22	0.9	0.1	1.0	5.5 2.22 22	6.3 3.33 22	11.8 3.75 22	51.1	6.1	8.3
2F	Mean 15.0 SD 1.76 N 19	14.2 1.84 19	0.7	0.2	0.9	6.8 1.95 19	6.5 1.98 19	13.3 1.95 19	51.4	6.5	6.4
3F	Mean 14.5 SD 2.84 N 23	13.8 2.21 23	0.6	0.2	0.7	6.7 2.39 23	6.4 1.67 23	13.1 2.64 23	49.9	6.5	5.9
4F	Mean 14.5 SD 2.58 N 20	13.8 2.00 20	1.1	1.0*	2.1	6.2 3.41 20	5.6 3.03 20	11.7 3.99 20	52.3	5.3	16.8*

Table 28 Summary of Mouse Placental Weight, Litter Weight, Litter Size and Fetal Weights

Group /Sex	Placental Weight	Litter Weight	Litter Size	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight
Statistical test:	Sh	Sh	Wi	Wi	Wi	Wi
1F	Mean 0.12 SD 0.029 N 22	13.08 4.314 22	11.82 3.750 22	1.13 0.114 22	1.09 0.096 20	1.11 0.104 22
2F	Mean 0.12 SD 0.012 N 19	14.81 1.948 19	13.32 1.945 19	1.14 0.077 19	1.09 0.084 19	1.12 0.076 19
3F	Mean 0.11 SD 0.014 N 23	13.89 2.820 23	13.09 2.644 23	1.09 0.134 23	1.05 0.136 23	1.07 0.130 23
4F	Mean 0.11 SD 0.013 N 20	11.73 3.940 20	11.70 3.988 20	1.02** 0.082 20	0.99** 0.086 20	1.00** 0.082 20

Tables from sponsor

9.3 PRENATAL AND POSTNATAL DEVELOPMENT

Study title: TMC435350: Pre- and Post-Natal Development Study in the CD Rat by Oral Gavage Administration

Study no.: TMC435-TiDP16-NC224/TOX9448

Study report location:



Conducting laboratory and location:

Date of study initiation:

GLP compliance: YES

QA statement: YES

Drug, lot #, and % purity: TMC435350, ZR494617PFA081, 100%

Key Study Findings

Maternal Findings:

- Two early deaths at 1000 mg/kg/day on Day 18 (after 12 doses) and on Day 10 (after 4 doses).
- Post-implantation survival index decrease at the mid- (94%, N=23) and high-dose (93%, N=22) compared with controls (96.2%, N=22).
- Significant body weight and body weight gain decreases were seen in maternal animals across dose groups through lactation (up to 40%).
- No maternal NOAEL based on adverse body weight decreases.

F1 Offspring Findings:

- Small build, significantly decreased body weight (~20% in offspring from high dose) and decreased body weight gain (up to ~40% in offspring from high dose).
- Kinked tail characterized as a build deformity; 4/24 mid-dose F1 males (from 3 different litters), 2/24 high dose F1 males (two different litters), 1/24 mid-dose F1 females, and 1/24 high dose F1 females. Historical data - 2/10 studies conducted between 2008-2011 show kinked tail in 1-2 rats (sponsor did not provide denominator). Kinked tail not considered teratogenic, since it was observed in isolation. The finding did not impact survival, behavior or mating or future reproductive performance.
- Delayed sexual maturation in F1 females and delayed righting reflex in F1 from mid and high dose may be related to the observed maternal toxicity.
- Decreased motor activity (rearing and ambulatory) in F1 offspring from high dose.
- No fetal NOAEL based on adverse body weight decreases in F1 from low dose.

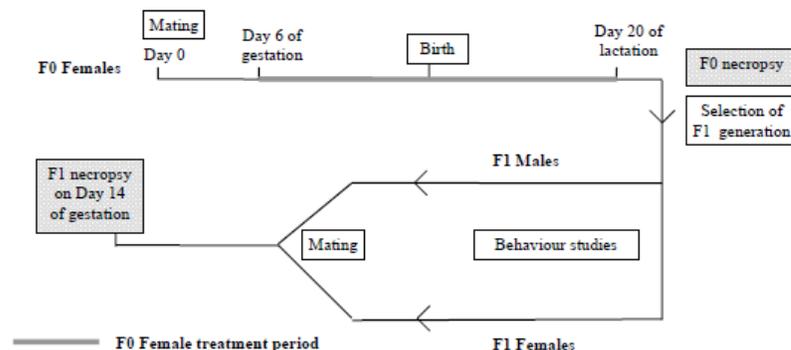
Methods

Doses: 150, 500 or 1000 mg/kg/day
 Frequency of dosing: Daily from Day 6 after mating to Day 20 of lactation
 Dose volume: 5 mL/kg/day
 Route of administration: Oral gavage
 Formulation/Vehicle: Polyethylene glycol 400 with 1.2 eq. Sodium hydroxide (NaOH) and 0.3 eq. Hydrochloric acid (HCl).
 Species/Strain: Crl:CD® (SD) rats

Number/Sex/Group: 24 female rats/group

Satellite groups: None.

Study design:



Deviation from study protocol: No impact deviations.

F₀ Dams

Survival: Three animals were sacrificed moribund, one vehicle control group female (No. 7 on Day 23 after mating) and two high dose females (No. 79 on Day 18 after mating and No. 93 on Day 10 after mating). The vehicle control female exhibited extended parturition with terminal signs of piloerection and pallor. The majority of this female's litter was dead. Two surviving offspring were unfed. Macroscopic examination of this female showed pale/inactive mammary tissue, an enlarged spleen and fetal material in the stomach. Examination of the uterus showed one dead male fetus and the placenta in the vagina, all implantation sites were empty.

Female No. 79 at 1000 mg/kg/day showed terminal signs of body weight loss from Day 14 of gestation, noisy/gasping breathing from Day 17 of gestation and on Day 18 piloerection and pallor in addition to the respiratory signs (after 12 days of treatment). Macroscopic examination showed abnormal GI contents (gaseous with yellow fluid). Uterine examination showed 13 implantation sites that grossly were normal.

No. 93 dosed at 1000 mg/kg/day showed terminal signs (Day 10 after 4 days of treatment) that included gasping/noisy breathing, hunched posture, pallor urine staining in the perigenital regions, red staining of the muzzle and forelimbs. Uterine examination showed 12 implantation sites that grossly appeared normal.

Clinical signs: Clinical signs included pale feces, noisy/gasping respiration, increased salivation and chin rubbing. Pale feces was seen in 5/24 high dose females during gestation and across dose groups during lactation: 2/24 at the low dose, 13/24 at the mid-dose and 22/24 females at the high dose during lactation. Noisy/gasping respiration was seen in 1/24 females at the mid-dose and 5/24 females at the high dose during gestation and in 1/24 females at the mid-dose

during lactation.

Clinical signs the sponsor attributed to dosing included chin rubbing and salivation during gestation and lactation.

The sponsor states that chin rubbing is often associated with increased salivation commonly seen in association with oral gavage administration and palatability of the test article formulation rather than pharmacology of the test material. During gestation the number of occasions of increased salivation was much more prominent in the high dose group. During lactation, the number of occasions of increased salivation was mainly seen in the mid and high dose groups.

The incidence of chin rubbing was observed in almost all treated animals (92%-100%) including 54% (during gestation) and 50% (during lactation) of the vehicle control animals. The number of occasions of chin rubbing increased with dose and was much higher in treated animals compared with control animals (3.8-14.5-fold higher during gestation and 11.6-25.8-fold higher during lactation). **These findings were not seen in the 3-month rat dietary feed study which may indicate that these clinical signs are related to oral gavage administration.**

Gestation Length, implantation sites, number of live litters and gestation index:

No treatment related changes in gestation length (22-23.5 days), number of implantation sites (13.9-14.7 implantations) number of live litters (22-24 litters) or gestation index were seen (96-100%). A summary of these F0 parameters is included in the sponsor's table below.

Table 29 Gestation Length and Gestation Index

Group	1	2	3	4
Compound	Control	TMC435350	TMC435350	TMC435350
Dose (mg/kg/day)	0	150	500	1000

Group	Number of pregnant animals	Gestation length (days)			Number of live litters born	Gestation index (%)
		22	22.5	23		
Statistical test: Lt						
1	24	n (%)	10 (42)	11 (46)	3 (13)	23 96
2	24	n (%)	5 (21)	10 (42)	9 (38)	24 100
3	24A	n (%)	8 (35)	9 (39)	6 (26)	23 96
4	22B	n (%)	6 (27)	14 (64)	2 (9)	22 100

A Percentage distribution of gestation lengths calculated from 23 animals - one pregnant female failed to litter
 B Excludes two pregnant females killed for welfare reasons on Day 10 or Day 18 after mating

A decrease in the post-implantation survival index (total # offspring

born/total # uterine implantation sites X100) was seen at the mid-dose (94%, N=23) and high-dose (93%, N=22) groups compared with controls (96.2%, N=22).

F0 Body weight:

Statistically significant body weight decreases were observed compared with controls on Day 20 of gestation at the high dose (5% decrease from controls). Body weight gain was decreased across dose groups on Gestation Days 17 to 20: 12% at the low dose, 10% at the mid-dose and 18% at the high dose as well as from Gestation Days 6 to 20 at the high dose (12%). During lactation, statistically significant decreases in body weight gain were seen across dose groups from Lactation Days 7 to 11: 40% at the low dose, 30% at the mid-dose, and 25% at the high dose. From Lactation Days 1 to 21, a statistically significant increase in body weight gain was seen at the mid-dose (24%) and the high dose (34%), potentially indicative of a compensatory body weight gain.

F0 Feed consumption:

Food consumption was statistically significantly decreased at various intervals during gestation and lactation. Food consumption decreases during gestation were mainly seen at the high dose with the exception of a slight decrease in mid-dose animals from Days 6-9 of 8%. High dose animals showed decreases at all measured intervals with the greatest decrease of 16% on Days 17-19.

During lactation decreases were seen at all dose groups at almost all of the measured time intervals. At the low dose, the decrease ranged from 7 to 13% with the greatest decrease seen on Days 11-13 and Days 14-17. At the mid-dose, the decrease ranged from 8 to 22% with the greatest decrease seen on Days 14-17. At the high-dose, the decrease ranged from 8 to 28% with the greatest decrease seen on Days 14-17.

F0 Uterine content:

F0 – Uterine contents in the F0 dams were evaluated in animals sacrificed moribund and are described under the Mortality section. There were no clear test article related differences in the numbers of implantations, live offspring on Day 1, postnatal survival to Day 4, postnatal survival to weaning and mean sex ratio on Day 1.

F0 Necropsy observation:

F0 – necropsy observations included dark (1/24 at low, mid and high) and pale areas in the lung (1/24 at mid and high), kidney pelvic dilatation (1/24 at low and mid-dose groups), and abnormal contents and distension of the cecum (no distension in treated group only in control group), duodenum, ileum, and jejunum (1/24 high dose group).

Dosing Solution

Analysis: The dose formulation analyses and stability analyses met the acceptance criteria specified in the protocol.

F₁

Generation

Survival and sex ratio: Survival of the F₁ generation from Day 1 to Day 4 (99.7% at control, 98.9% at low, 99.7% at mid, and 98.1% at high) and weaning (100% across vehicle and treated groups) did not show treatment related effects. There were no test article related effects on the mean sex ratio (49.6% at control, 47.7% at low, 49.3% at mid, and 50.6% at high). There were no test article related changes in the live birth, viability or lactation indices.

F1 Body weight: F1 offspring body weights showed dose and onset dependent decreases in body weight between 4-28 days of age in both males and females (culling occurred at 4 days of age). At the low dose and the mid-dose, statistically significant absolute body weight decreases were first observed on Day 11 (7.6 males at low 7.9% females at low, 15.3% males at mid, 15.1% males at mid) and continued to Day 28 with the exception of females at the low dose (6.9% males at low, 14.9% males at high, 12.5% females at high). At the high dose, body weight decreases started on Day 4 (before culling, -7.4% males, -9.9% females) until Day 28 (-24.1% males, -21.3% females). Body weight gain was statistically significantly decreased across dose groups. Consistent with the absolute body weights the greatest decreases occurred at the high dose at the earliest time interval. For the Day 1-21 interval (prior to weaning), body weight change decreases were 9.9% in males and 11.6% in females at the low dose, 21.4% in males and 21.0% in females at the mid-dose, and 36.6% in males and 36.3% in females at the high dose

Absolute body weights for selected F₁ generation animals after weaning/maturation were low on Day 1 (when F₁ offspring were ~35 days of age, body weights were as low as 21.5% in high dose males and 17.7% in high dose females compared to controls) and remained statistically significantly decreased during mating/pairing until termination in mid-dose (6.2%) and high dose males (11.6% on Day 53) as well as mid dose (4.8%) and high dose females (6.6%) through Gestation Day 14. Low dose males showed statistically significantly low body weights from Day 8 – Day 43.

Body weight gain was not significantly different in females during mating and gestation. Body weight gain in high dose males was

statistically significantly decreased in mid dose males up to Day 22 (4.9%) and in high dose males until Day 53 (7.1%).

Feed consumption: Not evaluated.

F₁ Clinical signs: The most notable clinical sign observed in the F₁ males and females was a dose dependent increase in kinked tail characterized as a build deformity. After mating, the clinical signs in females following gestation were similar with the exception of abnormal fur-staining of the head seen at a greater incidence than controls at the mid and high dose groups (10/24 at the mid dose and 6/24 at the high dose vs. 5/24 in controls).

F₁ Physical Development: Sexual maturation was statistically significant delayed in F₁ females at the mid-dose (36.0 days vs. 34.5 days in controls) and high-dose groups (36.3 days vs 34.5 days in controls). At the mid-dose group the mean body weight was similar to the control group (115 g vs 118 g in controls). At the high dose group, body weights were decreased compared with controls (108 g vs. 118 g) and correlated with the sexual maturity delay in females. Although not statistically significant, slight delays in sexual maturity in males were seen at the mid-dose (45.6 days vs. 44.4 days in controls) and high dose (45.8 days vs. 44.4 days in controls). Body weights were significantly decreased in high dose males (200 g vs. 227 g in controls).

Physical development: Pre-weaning reflex development examinations showed a statistically significant delay in the air righting reflex (assessed daily from Day 16 until achieved or until Day 21) in offspring from the 500 mg/kg/day group (17.6 days vs. 17.1 days for controls) and 1000 mg/kg/day group (17.8 days vs. 17.1 days for controls). A total of 6 offspring at 1000 mg/kg/day (5 offspring from one litter and 1 offspring from a second litter) failed the auditory function/startle response assessment on Day 20 but passed on Day 21. There were no effects on surface righting and visual function/pupil reflex in offspring following maternal treatment.

Motor Activity Decrease

Statistically significant decreases in motor activity on Day 22 included a lack of habituation for rearing activity (high beam scores) in F₁ males and females at the high dose, as well as decreases in ambulatory activity (low beam scores) in F₁ females from the high dose. When F₁ offspring were re-tested on Days 39-41, statistically significantly low measurements for rearing activity (high beam scores) in F₁ males at the high dose and a lack of habituation for ambulatory activity (low beam scores) in the F₁ females at the high

dose were observed.

F1 Locomotor Coordination (Accelerating rotarod) – no TMC435 related effects.

Other Macropathology: The most common macropathology finding in unselected F1 offspring was small build seen in 39 offspring from 7 litters at the high dose. Other macroscopic findings for unselected F1 offspring were seen in 1-2 animals in one litter and were not considered to be clinically significant. These findings included opaque eyes in one offspring in the control group, right dilated renal pelvis (1 offspring), absent tail (1 offspring), patchy coat (2 offspring) at the mid-dose and partially absent pinna at the high dose.

F1 - selected offspring, the findings included a dose dependent increase in kinked tail. A total of 6 F1 offspring (3/24 males and 1/24 females at the mid-dose, 1/24 males and 1/24 females at the high dose showed the finding and were all from different litters. Other findings appeared in only 1-2 offspring and no clear relationship to treatment was noted. A total of 8/96 (8.3%) males and females from 7 different litters showed the kinked tail physical deformity. Historical data provided by the sponsor showed the kinked tail finding in 1-2 rats in 2/10 rat peri- post-natal studies conducted between 2008-2011 (sponsor did not provide a denominator). Kinked tail finding was not considered to be teratogenic, since it was observed in isolation. The finding did not impact survival, behavior or mating or future reproductive performance.

F₂ Generation

Survival: No effect on embryo survival.

Body weight: Not evaluated.

External evaluation: No fetal abnormalities were observed.

Other: None.

Toxicokinetics: Toxicokinetics were not evaluated in the pivotal peri- post-natal study (Study NC224) but were evaluated in the preliminary peri- post-natal study (Study NC227) at the same doses used in the pivotal study of 150, 500 and 1000 mg/kg. The TK in maternal animals and F1 offspring are summarized in the sponsor's tables below.

Table 30 Maternal Toxicokinetics (Day 6 of lactation)

Dose (mg/kg/day)	Female		
	150	500	1000
C _{max} (ng/ml)	4993	6388	7495
T _{max} (h)	3	3	3
AUC _{0-24 h} (ng.h/ml)	38166	42502	56131

The maternal C_{max} and AUC exposures increased with dose less than dose-proportionally. The T_{max} was 3 h.

Table 31 F1 Offspring TK in Plasma and Liver

Dose (mg/kg/day)	150		500		1000	
	Plasma	Liver	Plasma	Liver	Plasma	Liver
C _{max} (ng/ml or ng/g)	104	254	341	781	399	1138
AUC _{0-24 h} (ng.h/ml or ng.h/g)	1897	5081	4142	15374	5559	22172

F1 offspring C_{max} and AUC plasma and liver exposures increased with dose approximately dose proportionally from 150-500 mg/kg/day and less than dose-proportionally from 500-1000 mg/kg/day. Group mean liver exposures in offspring were higher than plasma exposures (2.3-2.9X by C_{max} and 2.7-4.0X by AUC). The maternal to pup AUC group mean ratios were 20X at 150 mg/kg/day and 10X at 500 and 1000 mg/kg/day. The maternal to pup AUC ratio decreased with increasing dose from 150-500 mg/kg/day and appeared to saturate between 500-1000 mg/kg/day.

10 SPECIAL TOXICOLOGY STUDIES

Reviews on special toxicology studies by Christopher Ellis, PhD are included below:

Local tolerance:

In vitro studies:

- Bovine corneal opacity-permeability assay was performed by applying a 20% suspension of TMC435350 for 4h (NC131). Small increase in opacity and permeability was observed and thus may be a mild eye irritant.
- TMC435350 was shown to be phototoxic in the Balb/c 3T3 mouse fibroblast assay (NC216).

In vivo studies:

- TMC435350 was negative in the local lymph node (skin sensitization) assay after topical administration (0, 2.5, 5, 10% w/v) in mice (n=4) (NC174).
- No skin reactions were observed in the rabbit (n=3) primary skin irritation test (NC203). TMC435350 was applied for 4h and observed at 1, 24, 48 & 72h time points.

Other: *In vitro* hepatocyte cytotoxicity was evaluated by LDH release, neutral red uptake and ATP content determinations. ED₅₀ values were similar in all species tested; rat, 3.5 to 10 µg/ml, dog, 2.8 to 6.6 µg/ml, cynomolgus monkey, 8.2 to 19.1 µg/ml and human, 4.1 to 25.9 µg/ml (NC114). **Conclusion:** Although assay is not performed in the presence of serum protein (so *in vivo* exposure could be somewhat lower), the liver to plasma ratio in rat biodistribution studies is 39 and concentrations within this cytotoxic range are reached as shown in both animal and human PK studies, indicating that liver hepatocytes are likely a target of cytotoxicity *in vivo*. Toxicity was confirmed in repeat-dose studies (see above).

11 INTEGRATED SUMMARY AND SAFETY EVALUATION

The nonclinical safety package for TMC435 was adequate and included pharmacology, safety pharmacology, ADME, general toxicology, reproductive toxicology, and genotoxicity studies. Carcinogenicity studies were not required based on the 12 week indication and negative genotoxicity studies. Nonclinical studies were conducted across various species. Embryofetal studies were conducted in the mouse and rat because the rabbit had poor bioavailability (2.5%). The rat and dog were evaluated in the pivotal toxicology studies and appeared to be relevant species based on overall similar PK and metabolite profiles compared with human.

TMC435 was evaluated in respiratory, central nervous system (CNS), and cardiovascular safety pharmacology studies. No effects on the respiratory system were noted. In the rat, neurological effects included diminished alertness, narrowing of the palpebral fissure, and, in one rat at the high dose of 500 mg/kg, clonic jaw muscle contraction. Following a single dose in the mouse, a decrease in general activity, narrowing of the palpebral fissure, and piloerection were seen at 1000 and 2000 mg/kg. Treatment-related CNS effects have not been observed in the clinic to date. A delay in gastric emptying was observed in rats 1 hr following oral doses of TMC435 from 160 mg/kg-640 mg/kg. In evaluations of cardiac toxicity, *in vitro* studies showed a potential inhibitory effect on membrane Na⁺ channel current (hH1a, human heart sodium channel) at concentrations as low as 70 ng/ml. In the isolated Langendorff-perfused rabbit heart assay, proarrhythmic effects appeared to correlate with high levels of drug accumulation in the heart (median 597 µg/g). The *in vivo* dog studies showed increased vascular resistance, decreased heart rate, decreased cardiac output as well as an increase in the RR-interval. The potential for cardiovascular toxicity is further discussed below. Cardiovascular toxicity has not been observed in clinical trials to date.

The bioavailability of TMC435 was moderate to high across tested species (19-72%) with the exception of the rabbit (2.5%). TMC435 is primarily metabolized by CYP3A4 with unchanged drug as the predominant moiety in plasma and no major (10% or greater) metabolites identified. TMC435 is highly bound to albumin and predominantly distributes to the liver and gastrointestinal tract. TMC435 is primarily excreted in the feces across species.

Decreased exposure following repeat dosing was seen in toxicology studies in the mouse (2-week oral gavage, 2-week dietary feed, 3-month dietary feed study), the rat

(13-week dietary feed, 1-month oral gavage and 6-month oral gavage) and the dog (6-month oral gavage, 39-week oral gavage). In the dog, the decrease was as high as 55% in female dogs between Day 90 and Day 273 in the 39-week study. Decreases in TMC435 exposure with repeat dosing was not observed in clinical trials.

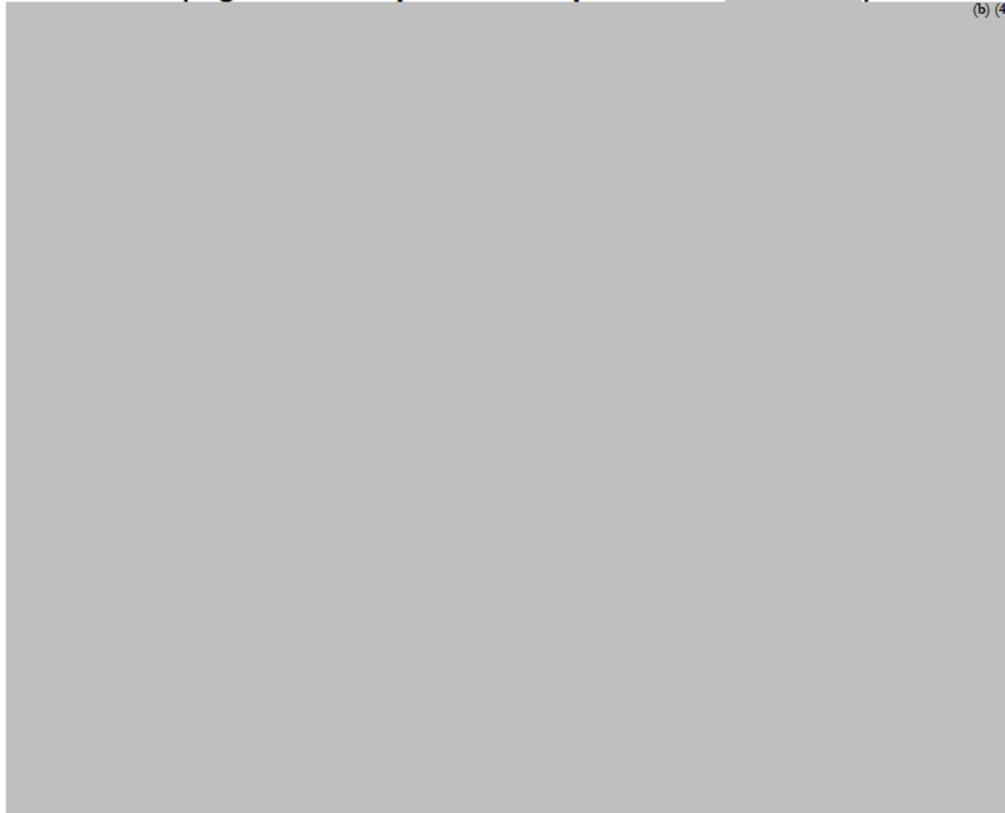
Enzyme induction in nonclinical species may be a factor in the reduced exposure. Although induction of CYP1A2 or CYP3A4 was not seen in human hepatocytes, induction of various CYPs was observed in *ex vivo* liver microsomes from studies in the mouse (CYP4A, CYP3A, CYP2E, and thyroxine UDPGT), rat (CYP2B1, CYP2E1, CYP3A4) and dog (CYP4A and CYP1A). The sponsor could not identify the mechanism of the reduced exposure following repeat dosing in nonclinical species but did note that potential mechanisms could include an effect of TMC435 on absorption as well as on GI and hepatic efflux transporters (P-gp/MDR1, MRP2 and Bcrp) and hepatic uptake transporters (OATP1B1, OATP1B3, and OATP2B1).

General toxicology studies were conducted in the mouse, rat, dog and monkey. The major target organs identified in the TMC435 nonclinical studies include the gastrointestinal tract and the liver, consistent with the clinical trial safety profile. Although there are no safety margins for these toxicities, the liver and GI have been identified as target organs in the clinical trials. The heart was identified to be a potential target organ (acute endocardial and myocardial necrosis) in the dog at high doses (~28X the clinical AUC); no cardiac safety signals have been identified in the clinical trials. Toxicities observed in the liver, GI/pancreas, heart, and adrenals are further discussed below.

Liver Toxicity

The liver was a target organ across species and an identified target organ in clinical trials. Overall, hepatocellular necrosis was observed in dogs, and centrilobular hepatocellular hypertrophy was seen in rats and mice. The hepatocellular hypertrophy may indicate induction of liver enzymes. Clinical chemistry changes across species included increases in ALT, ALP, AST, and bile acids, which correlated with the liver histopathology findings. Additional clinical chemistry changes included decreased cholesterol, triglycerides, and total protein which may be related to the observed liver toxicity.

The increase in bilirubin and bile acids is likely due to TMC435 inhibition of bilirubin uptake (OATP1B1), efflux (P-gp, MRP2) and bile salt (BSEP, NTCP) hepatocyte transporters as summarized in the sponsor's schematic below (Figure 3).

Figure 3 Schematic of Bilirubin Clearance by Liver Cells and Potential Impact of TMC435 (Figure from Sponsor Adapted from (b) (4))

Minimal liver canalicular cholestasis was seen in the 2-week dog study at a low incidence in 1/3 high dose males and 1/3 high dose females in moribund condition at exposures ~28X over the anticipated clinical exposure; this study achieved the highest TMC435 exposures following oral dosing in the dog. Interestingly, hepatocellular necrosis was observed in the 6-month dog toxicity study but not in the 39-week dog toxicity study. The lack of liver toxicity may be related to the lower exposures in the 39-week dog study compared with the 6-month dog study; the females in the 6-month study showed 5-6X higher AUC values than females in the longer duration, 39-week study. Recovery was not evaluated in the 6-month dog study but was evaluated in the 39-week dog study. In the 39-week dog study, liver related clinical chemistry changes were seen at the end of the treatment phase and appeared to recovery following the 13 week recovery phase. No significant liver histopathology was observed at either the end of the treatment or recovery phases.

Gastrointestinal/Pancreas Toxicity

Significant body weight decreases >10% were seen across species and study durations which may be related to the GI effects of the drug. The most severe body weight decreases were seen in the 3-month mouse dietary feed study after only 1 week of dosing (decrease of 155% in males and 113% in females compared to controls); the decrease was associated with a decrease in food consumption.

GI effects were mainly seen in the small intestine across species. In dogs, dilatation of the crypts and lacteals of the small intestine were seen. In rats and mice, GI distension, delayed gastric emptying and vacuolation of the apical enterocytes were seen. Finally in rats and mice, the pancreas appeared to be a target organ with decreased zymogen/basophilia, vacuolation as well as changes in amylase and lipase levels. In dogs the pancreatic findings included inflammation and mononuclear infiltrates.

One potential mechanism that may be related to the small intestine, pancreas and delayed gastric emptying observations is the inhibition of cholecystokinin (CCK) by TMC435. TMC435 was shown to inhibit CCK by 60% in an in vitro receptor inhibition screening assay; this result was not reproduced in a second receptor screening assay. CCK is involved in the digestion of fat and protein. High levels of CCK can delay gastric emptying. However, fat absorption could also be impacted by an effect on bile salts by TMC435. This mechanism may explain the vacuolation of the apical enterocytes, delayed gastric emptying, and pancreas findings in rats and mice. The sponsor argues that the majority of the GI effects are most likely related to the local intestinal effects of high exposure and duration of local physical contact between TMC435 and the small intestine wall rather than an effect on CCK.

Cardiovascular Toxicity

The cardiovascular system appeared to be a target organ in dogs at a high exposure of approximately 28X the anticipated clinical exposure. Clinical trials have not identified a cardiovascular safety signal to date. Cardiovascular toxicity was seen in the dog IV safety pharmacology study (increased vascular resistance, decreased heart rate, decreased cardiac output as well as an increase in the RR-interval), and the 2-week oral dog (acute endocardial and myocardial necrosis, restricted to the left ventricular subendocardial area). Findings of unknown clinical relevance include PR prolongation in the 39-week oral dog study (seen at 26 weeks but not at 13 or 39 weeks) at an exposure lower than the clinical AUC. In the 28 day monkey study, inflammatory cell foci were seen in the heart in 5/5 treated monkeys at a TMC435 exposure 11.5X the clinical AUC; there were no associated changes in ECGs, cardiac echocardiography, or cardiac troponin-T (quick test) in the monkey. The in vitro safety pharmacology studies showed a potential inhibitory effect on the membrane Na⁺ channel current (hH1a, human heart sodium channel) at concentrations as low as 70 ng/ml. In the isolated Langendorff-perfused rabbit heart assay, proarrhythmic effects appeared to correlate with high levels of drug accumulation in the heart (median 597 µg/g). In conclusion, potential cardiovascular toxicity may be a risk in patients who have higher exposures of TMC435.

Adrenal Toxicity

The adrenal glands appeared to be a target organ of toxicity with organ weight changes and minimal focal cortical hyperplasia in the rat, vacuolization in the dog and mouse, reduced X zone vacuolation, increased prominent X zone, and cortical atrophy in female mice. Adrenal gland-related toxicities have not been reported in the clinic.

TMC435350 was not mutagenic in the Ames assay or the mammalian forward mutation assay in mouse lymphoma cells (L5178Y), and was not clastogenic or aneuploidic in vivo in the mouse micronucleus test.

Carcinogenicity studies were not performed because the proposed indication is 12 weeks. Additionally, TMC435 was not associated with hyperplasia or potential signals of carcinogenicity.

TMC435 findings in the rat fertility study included an increase in post-implantation loss, and a decrease in body weight gain in females during pregnancy. Three males showed 100% static sperm that was associated with small testes and epididymides (unilateral and bilateral) and had a negative impact on pregnancy outcome. The sponsor argued that the static sperm finding was incidental since it has been seen in control animals in other studies; however, the incidence of the finding in treated groups within the current study was 4-8X higher than the historical control incidence provided by the sponsor.

Teratogenic findings of exencephaly and protruding tongue were observed in the pilot mouse embryofetal study. The teratogenic findings were not reproduced in the pivotal mouse study which may be explained by the following: 1) the pivotal study was conducted at a different laboratory than the pilot study, 2) the mice were from a different source, 3) the testing conditions were different between the two laboratories and finally, 4) the dose volume was reduced. TMC435 findings in the pivotal mouse embryofetal study included two maternal deaths at 1000 mg/kg, high post-implantation loss, decreased fetal weight and an increase in skeletal variations. Teratogenicity was not observed in the rat embryofetal toxicity study.

In the rat peri- post-natal study, there were two early maternal rat deaths at the high dose of 1000 mg/kg/day. The post-implantation survival index was decreased in TMC435-treated maternal rats. Significant body weight and body weight gain decreases were seen in maternal animals through lactation (up to 40%). In the F1 offspring, small build was seen in many of the fetuses as well as significantly decreased body weight (~20% in offspring from high dose) and body weight gain (up to ~40% in offspring from high dose). Offspring from the mid dose of 500 mg/kg and the high dose of 1000 mg/kg/day showed delayed righting reflex and delayed sexual maturation in the females which may be secondary to the observed maternal toxicity. Finally, decreased motor activity (rearing and ambulatory) in the F1 offspring from the high dose indicated physical development effects of treatment with TMC435.

The totality of the reproductive toxicity effects of TMC435 in maternal animals (mortality and post-implantation loss), the fetus (adverse body weight decreases, skeletal variations), and developing offspring (adverse body weight decrease, small size, motor activity decrease) at exposures similar to clinical exposures lead us to conclude that a Pregnancy Category C would be more appropriate than a (b) (4) which was originally proposed by the sponsor.

TMC435 may be a mild eye irritant as observed in the bovine corneal opacity-permeability assay and was shown to be phototoxic in the in the Balb/c 3T3 mouse fibroblast assay; this is consistent with clinical findings of rash and photosensitivity. TMC435350 was negative in the skin sensitization assay and skin irritation test.

In summary, the TMC435 nonclinical package adequately characterized the nonclinical safety pharmacology, PK/ADME, toxicology, reproductive toxicology, and genotoxicity of simeprevir. The identified target organs in the nonclinical studies, the liver and the GI tract, are consistent with the observed clinical trial safety profile. Potential cardiac toxicity may be an issue at higher doses. The risk of reproductive toxicity with TMC435 will be addressed and communicated in labeling. Although exposure margins obtained in the nonclinical studies were generally similar to or lower than the anticipated clinical exposures (Table 32, Table 33), this appears to be acceptable based on the nonclinical findings in the liver and the GI tract. In conclusion, simeprevir is approvable from a nonclinical perspective.

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Table 32 Safety Margins for TMC435/Simeprevir Across Pivotal Studies

<u>Species</u>	<u>Duration/ Study</u>	<u>Route</u>	<u>NOAEL (mg/kg)</u>	<u>AUC* (ng*hr/ml)</u>	<u>Exp Mult AUC¹</u>
Mouse	3 months	Diet	500	50,800	0.9
Rat	3 months	Diet	None, 500	40,757 ²	0.7²
Rat	6 months	Gavage	150	26,850	0.5
Dog	2 weeks	Gavage	10	72,850	1.3
Dog	6 months	Gavage	15	74,800	1.3
Dog	39 weeks	Gavage	5	6,823	0.1
Mouse	Embryofetal	Gavage	500	221,000	3.9
Rat	Fertility	Gavage	None, 50	10,575 ³	0.2³
Rat	Embryofetal	Gavage	500	29,311	0.5
Rat	Peri- Post-Natal	Gavage	None, 150	38,166 ²	0.7²

*Male and female AUC values were averaged for the general toxicology studies.

¹Clinical AUC 57,460 ng*hr/ml from pooled Phase 3 trials following 150 mg daily dose for 12 weeks.

²For studies with no identified NOAEL, the lowest dose exposure values are provided for reference.

³TK was not measured in the rat fertility study. Mean exposure values from the 3-month interim rat study were used to calculate approximate safety margins.

Table 33 Comparison of Steady State Plasma Exposures Across Preclinical Species Compared to Human, Table from Sponsor

Species, Study (Study No.)	Dose (mg/kg/day)	Day	C _{max} (µg/mL)		AUC (µg.h/mL)		Animal/Human Exposure Ratios			
			M	F	M	F	C _{max}		AUC	
							M	F	M	F
Mouse, 3 mo RD/gavage (TMC435-NC206)	150	90	10.1	15.0	50.9	61.2	2.3	3.4	0.9	1.1
	500	90	17.6	15.0	117	78.8	4.0	3.4	2.0	1.4
	2000/1000 ^a	90	14.9	14.3	122	141	3.4	3.3	2.1	2.5
Mouse, 13 we RD/diet (TMC435-NC233)	500	88	-	-	33.3	68.3	-	-	0.6	1.2
	2000	88	-	-	66.7	180	-	-	1.2	3.1
Mouse, 13 we RD/diet (TMC435-NC233)	3000	87	-	-	189	143	-	-	3.3	2.5
Rat, 1 mo RD/gavage (TMC435-NC177)	50	28	1.76	2.57	8.80	12.6	0.4	0.6	0.2	0.2
	150 (NOAEL)	28	3.82	3.84	22.6	26.9	0.9	0.9	0.4	0.5
	500	28	4.04	4.74	33.7	42.0	0.9	1.1	0.6	0.7
Rat, 6 mo RD/gavage (TMC435-NC179)	50	181	2.11	2.39	8.32	12.7	0.5	0.5	0.1	0.2
	150	181	3.65	4.76	20.9	32.8	0.8	1.1	0.4	0.6
	500 (NOAEL)	181	4.08	7.22	30.2	52.7	0.9	1.6	0.5	0.9
Rat, 13 we RD/diet (TMC435-NC244)	500	88	-	-	23.8	57.8	-	-	0.4	1.0
	1000	88	-	-	28.3	55.4	-	-	0.5	1.0
	2000	88	-	-	26.8	99.3	-	-	0.5	1.7
Dog, 2 we RD/gavage (TMC435-NC161)	10 (NOEL)	14	13.8	14.3	73.3	72.4	3.1	3.3	1.3	1.3
	40	14	71.9	36.5	876	520	16.4	8.3	15.2	9.0
	160/120 ^b	14	81.2	93.3	1510	1660	18.5	21.2	26.3	28.9
Dog, 1 mo RD/gavage (TMC435-NC175)	10 (NOEL)	27	4.02	4.57	15.5	18.6	0.9	1.0	0.3	0.3
	30	27	29.5	40.5	201	374	6.7	9.2	3.5	6.5
	90	27	93.5	94.8	1478	1710	21.2	21.6	25.7	29.7
Dog, 6 mo RD/gavage (TMC435-NC226)	5	177	2.42	2.92	8.98	10.4	0.6	0.7	0.2	0.2
	15 (NOAEL)	177	14.3	17.2	69.9	79.7	3.3	3.9	1.2	1.4
	45	177	41.0	72.7	475	833	9.3	16.6	8.3	14.5
Dog, 39 we RD/gavage (TMC435-NC207)	5	273	1.26	2.25	4.34	9.31	0.3	0.5	0.1	0.2
	15 (NOAEL)	273	10.5	17.2	47.4	70.1	2.4	3.9	0.8	1.2
	45	273	36.1	24.9	297	152	8.2	5.7	5.2	2.6
Mouse, embryo-fetal development/gavage (TMC435-NC189)	150	GD15	-	13.1	-	90.7	-	3.0	-	1.6
	500 (NOAEL)	GD15	-	17.5	-	221	-	4.0	-	3.8
	1000	GD15	-	25.0	-	352	-	5.7	-	6.1
Rat, embryo-fetal development/gavage (TMC435-NC188)	50	GD17	-	2.24	-	16.4	-	0.5	-	0.3
	150	GD17	-	3.45	-	28.3	-	0.8	-	0.5
	500 (NOAEL)	GD17	-	3.59	-	29.3	-	0.8	-	0.5
Human ^c	150	overall	4.39		57.5		-		-	

^a 2000 mg/kg/day until Day 7 and 1000 mg/kg/day from Day 8

^b males/females

^c Exposure data in HCV-infected patients after treatment with TMC435 at 150 mg q.d. for 12 weeks were derived from C205 Phase IIb trial (C_{max}) and from pooled C208, C216 and HPC3007 Phase III trials (AUC).

F = female; GD = gestation day; M = male; NOEL = no observed effect level; NOAEL = no observed adverse effect level

12 APPENDIX/ATTACHMENTS

The following are the repeat-dose toxicity reviews by Christopher Ellis, PhD.

Repeat-dose toxicity:

Study title:	<u>2-Week Repeated Dose Oral Toxicity Study of TMC435350 in the Rat</u>
Study #:	NC144
CTD location:	4.2.3.2
Conducting laboratory and location:	Johnson and Johnson Pharmaceutical Research & Development, Global Preclinical Development Beerse site
Date of study initiation:	July 04, 2006
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	TMC435350, ZR494617PFA011, 92.7%

Key findings: The NOAEL is 120 mg/kg/day based on changes in liver-related parameters, including reduced cholesterol, total protein and increased ALT, at high dose.

Methods:

Doses:	0, 40, 120, 360 mg/kg/day
Species/strain:	SPF Sprague-Dawley rats
#/sex/group (main study):	10
Route/formulation/volume/infusion rate:	oral gavage/PEG400, 2.5% Vit E-TPGS, NaOH/5 ml/kg/day/NA
#/sex/group (satellite for TK or recovery):	3 (TK)
Age:	~6 wks
Weight:	133 to 220g
Sampling times:	0/1&14/15d(TK),14/15(Hem/Coag/Urin/CC)

Results:

Mortality (2x/day): None

Clinical signs (2x/day): No test-article related changes noted.

Body weights (1x/wk): No test-article related changes noted.

Food consumption (1x/wk): No test-article related changes noted.

Ophthalmoscopy: No test-article related changes noted.

Hematology: No test-article related changes noted.

Coagulation: No test-article related changes noted.

Clinical chemistry:

- minor ↓ total protein in MD & HD (most 8% in ♀ HD)
- cholesterol ↓21% (♀ HD)
- ALT 30%↑ (♀ HD)

Urinalysis: No test-article related changes noted.

Gross pathology: No test-article related changes noted.

Organ weights: No test-article related changes noted.

Histopathology: [Adequate Battery: yes (X), no ()]; Peer review: yes (), no (X)]

- Minimal focal liver necrosis (1/10 ♂ & ♀ HD)-observed in historical controls
- Reduced pigmentation in popliteal lymph nodes (♀)
- Minimal granulocytic infiltrate in rectum (2 ♀ HD)

Toxicokinetics: Liver/plasma ratios range from 54 to 120 (in all dose groups ♂ & ♀)

		40 mg/kg/day		120 mg/kg/day		360 mg/kg/day	
		Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
C _{max}	Male	2.03	2.41	2.97	2.24	3.48	3.22
	Female	1.82	2.31	2.46	2.88	4.05	3.69
AUC	Male	12.6 ¹	15.9	29.2	21.7	40.7	29.5
	Female	16.7	18.5	28.2	28.7	49.3	41.7

¹ AUC_{0-3h}

Units: C_{max}=µg/ml & AUC=µg.h/ml (table from sponsor)

Study title:	<u>1-mos Repeated Dose Oral Toxicity Study of TMC435350 in the Rat with a 1-mos Recovery Period.</u>
Study #:	NC177
CTD location:	4.2.3.2
Conducting laboratory and location:	Johnson and Johnson Pharmaceutical Research & Development, Global Preclinical Development Beerse site
Date of study initiation:	January 09, 2007
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	TMC435350, ZR494617PFA031, 94.9%

Key findings: The NOAEL is 150 mg/kg/day based on changes in urine pH, color and presence of occult blood, and in liver-related parameters, including increased ALT, at high dose. No test-article related changes were noted in recovery group.

Methods:

Doses:	0, 50, 150 or 500 mg/kg/day
Species/strain:	SPF Sprague-Dawley rats
#/sex/group (main study):	10
Route/formulation/volume/infusion rate:	oral gavage/PEG400/5 ml/kg/day/NA
#/sex/group (satellite for TK or recovery):	6-3 groups (TK), 5-2 groups (recovery)

Age:	~6 wks
Weight:	126 to 224g
Sampling times:	0/1&28d(TK);14,28,56d(Hem/Coag/Urin/CC)

Results:

Mortality (2x/day): None

Clinical signs (2x/day): No test-article related changes noted.

Body weights (1x/wk): No test-article related changes noted.

Food consumption (1x/wk): No test-article related changes noted.

Ophthalmoscopy: slight fundus hyperreflectivity observed in one eye (1♂ HD)-also observed in historical controls

Hematology: No test-article related changes noted.

Coagulation: No test-article related changes noted.

Clinical chemistry:

- ↓3-6% total protein (& albumin) (♀&♂ HD)-14/28d
- cholesterol ↑34%, triglycerides ↑27% (♀ HD)-14d
- cholesterol ↑22%, triglycerides ↑24%, BUN ↓20% (♂ HD)-28d
- ALT 43-44%↑ (♀&♂ HD)-14d; ALT 37%↑ (♀ HD)-28d
- No test-article related changes noted in recovery group.

Urinalysis:

- ↑369% squamous epithelial cells (♂ HD)-14d (likely skin contamination)
- Color ↑33-51% (♂&♀ HD-14d); ↑27(NS)-50% (♂&♀ HD-28d)
- pH 6.6+/-0.1 (♂ HD-14d) & 6.5+/-0.1 (♂ HD-28d) versus 7.1+/-0.1 (control)
- Occult blood ↑444-765%, RBC ↑63(NS)-369% (♂&♀ HD)-28d
- Recovery group obs: pH 6.9+/-0.1 (♂ HD) versus 8.0+/-0.1 (control) (recovered?)
- Note: Color, pH & occult blood also outside historical norms.

Gross pathology: No test-article related changes noted.

Organ weights: adrenal gland 17%↑ (♂ HD)-not different than control after recovery period

Histopathology: No test-article related changes noted. All controls, HD, & animals with a necropsy finding examined. [Adequate Battery: yes (X), no (); Peer review: yes (), no (X)]

Toxicokinetics:

	50 mg eq./kg/day		150 mg eq./kg/day		500 mg eq./kg/day	
	Female	Male	Female	Male	Female	Male
Single dose (day 0/1)						
C_{max} (ng/ml)	3150	2660	4520	4480	5900	5170
AUC_{0-24h} (ng.h/ml)	26000	18600	41000	35600	66200	46400
1-Month Repeated dose (day 28/29)						
C_{max} (ng/ml)	2570	1760	3840	3820	4740	4040
AUC_{0-24h} (ng.h/ml)	12600 ¹⁾	8800 ¹⁾	26900	22600	42000	33700

¹⁾ AUC_{0-8h}

Table from sponsor

Study title:	<u>6-mos Repeated Dose Oral Toxicity Study of TMC435350 in Rat w/ 3 mos interim kill: interim report</u>
Study #:	NC179
CTD location:	4.2.3.2
Conducting laboratory and location:	Johnson and Johnson Pharmaceutical Research & Development, Global Preclinical Development Beerse site
Date of study initiation:	June 14, 2007
GLP compliance:	Yes
QA report:	Yes-interim report
Drug, lot #, and % purity:	TMC435350, ZR494617PFA051, 95.9%

Key findings: The NOAEL is 150 mg/kg/day (sponsor: 500 mg/kg/day) based on changes 1) in urine pH and color, 2) in liver-related parameters, including reduced total protein and increased ALT, and 3) a reduction in reticulocytes and increase in neutrophils observed at the high dose.

Methods:

Doses:	0, 50, 150 or 500 mg/kg/day
Species/strain:	SPF Sprague-Dawley rats
#/sex/group (main study):	30 (10 interim sacrifice)
Route/formulation/volume/infusion rate:	oral gavage/PEG400/5 ml/kg/day/NA
#/sex/group (satellite for TK or recovery):	6 (TK), no recovery animals
Age:	~6 wks
Weight:	148 to 228g
Sampling times:	1&90d(TK);1&3mos (Hem/Coag/Urin/CC)

Results:

Mortality (2x/day): None due to test article. Mortality observed in 10/240 animals thought to be due to gavage procedure/formulation in lungs (5♂&5♀; 2 cont., 1 LD, 6 MD & 1 HD).

Clinical signs (2x/day): No test-article related changes noted.

Body weights (1x/wk): No test-article related changes noted.

Food consumption (1x/wk): No test-article related changes noted.

Ophthalmoscopy: No test-article related changes noted (control versus HD-3 mos).

Hematology:

- Reticulocytes (% & #) ↓10-16% (♂&♀ HD-1 mos); ↓21-23% (♀ HD-3 mos)
- Neutrophils (#) ↑20-27% (♂ MD&HD-1 mos); ↑30% (♀ HD-3 mos)
- Changes in reticulocytes & neutrophils outside historical norms for ♀ HD-3 mos.

Coagulation: APTT small ↓ in ♂ (HD-1&3 mos) & ♀ (HD-3 mos)

Clinical chemistry:

- ↓7-8% total protein (& albumin) (♀&♂ HD)-1&3 mos
- ALT 31-39%↑ (♂&♀ HD-1 mos); 21-38%↑ (♂&♀ HD-3 mos)
- Small ↓ in Mg, K (♂&♀ HD-1&3 mos); small ↑ phosphorus (♀ HD-1 mos)

Urinalysis:

- Color ↑85-255% (♀&♂ HD-1mos); ↑28-55% (♂&♀ HD-3mos)
- pH 6.6+/-0.0 (♂ HD-1mos) & 6.4+/-0.1 (♀ HD-1mos) versus 7.0/6.7+/-0.1 (cont)
- Note: Color, pH outside historical norms.

Gross pathology: No test-article related changes noted.

Organ weights: adrenal gland 20-23%↑ (abs&%) (♂ HD)

Histopathology: No test-article related changes noted. [Adequate Battery: yes (X), no ()]; Peer review: yes (), no (X)]

Toxicokinetics:

Day	50 mg/kg/day				150 mg/kg/day				500 mg/kg/day			
	M		F		M		F		M		F	
	0	90	0	90	0	90	0	90	0	90	0	90
C_{max} (ng/ml)	2050	1640	2440	3080	3580	3730	5180	4340	5150	3210	5700	5750
$AUC^{1)}$ (ng.h/ml)	13000 ²⁾	9450	13800 ²⁾	11700	32900	22500	37100	25600	41300	30200	62800	46400

¹⁾ AUC_{0-INF} (SD) or AUC_{0-24h} (RD)

²⁾ AUC_{0-3h}

Table from sponsor

Repeat-dose toxicity:

Study title:	<u>6-mos Repeated Dose Oral Toxicity Study of TMC435350 in Rat w/ 3 mos interim kill: terminal report</u> (Note: interim report submitted with original IND).
Study #:	NC179
CTD location:	4.2.3.2
Conducting laboratory and location:	Johnson and Johnson Pharmaceutical Research & Development, Global Preclinical Development Beerse site
Date of study initiation:	June 14, 2007
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	TMC435350, ZR494617PFA051, 95.9%

Key findings: The NOAEL is 150 mg/kg/day (sponsor: 500 mg/kg/day) based on mildly adverse changes in urine color, in liver-related parameters (including reduced total protein and increased ALT), a reduction in reticulocytes, and increased blood glucose observed at the high dose. These changes, although potentially adverse, were not severe and were not exacerbated over the time course of study. Although no recovery animals were included in this study, thereby making it impossible to determine conclusively if effects were reversible, similar test article related observations were noted previously in a 1 month study that did recover during a 1 month recovery period (study #NC177).

Methods:

Doses:	0, 50, 150 or 500 mg/kg/day
Species/strain:	SPF Sprague-Dawley rats
#/sex/group (main study):	30 (10 interim sacrifice)
Route/formulation/volume/infusion rate:	oral gavage/PEG400/5 ml/kg/day/NA
#/sex/group (satellite for TK or recovery):	6 (TK), no recovery animals
Age:	~6 wks
Weight:	148 to 228g
Sampling times:	1, 90 & 181d (TK); 1, 3 & 6 mos (Hem/Coag/Urin/CC)

Results:

Mortality (2x/day): None due to test article. Mortality observed in 15/240 animals (7 ♂ & 8 ♀; 4 cont., 2 LD, 6 MD & 3 HD) thought to be due to gavage procedure/formulation in lungs as a result of high viscosity of the vehicle. See appendix for individual animal observations.

Clinical signs (2x/day): No test-article related changes noted.

Body weights (1x/wk): No test-article related changes noted.

Food consumption (1x/wk): No test-article related changes noted.

Ophthalmoscopy: Note: Only control and HD animals examined (3 & 6 mos). No test-article related changes noted.

Hematology:

- Reticulocytes (% & #) ↓10-16% (♂&♀ HD-1 mos), ↓21-23% (♀ HD-3 mos), ↓16-26% (♀ LD, MD & HD-6 mos) & ↓16% (♂ HD-6 mos)
- Neutrophils (#) ↑20-27% (♂ MD & HD-1 mos) & ↑30% (♀ HD-3 mos)
- Monocytes (#) ↓26% (♀ HD-6 mos)
- Changes in reticulocytes & neutrophils outside historical control range for ♀ HD-3 & 6 mos.

Coagulation: small ↓ in APTT (♂ HD-1, 3 & 6 mos & ♀ HD-3 & 6 mos)

Clinical chemistry:

- total protein (& albumin) ↓7-8% (♀&♂ HD)-1 & 3 mos & ↓12 & 6% (♀&♂ HD)-6 mos
- ALT 31-39%↑ (♂&♀ HD-1 mos) & 21-38%↑ (♂&♀ HD-3 mos)
- Mg ↓6-8% (♀&♂ HD)-1 mos & ↓5% (♂ HD)-3 mos; phosphorus ↑9% (♀ HD)-1 mos)
- Glucose ↓5-9% (♀&♂ HD)-3 mos & ↓9-13% (♀&♂ MD & HD)-6 mos

Urinalysis:

- Color ↑85-255% (♀&♂ HD-1 mos); ↑28-55% (♂&♀ HD-3 mos); ↑30-44% (♀&♂ HD-6 mos)
- pH 6.6+/-0.0 (♂ HD-1 mos) & 6.4+/-0.1 (♀ HD-1 mos) versus 7.0/6.7+/-0.1 (control)
- RBCs present (♂ HD-6 mos) 0.31+/-0.12 versus 0.00 in control
- Note: Color, pH outside historical norms.

Gross pathology: Pale discoloration of liver in 5/20 (♂ HD-6 mos)

Organ weights: adrenal gland 20-23%↑ (abs & %) associated with minimal focal cortical hyperplasia in 1/10 (♂ HD)-3 mos Note: 1/20 (♂ HD)-6 mos also with this observation.

Histopathology: Note: Only examined in control and high dose unless further examination warranted due to gross pathology findings. [Adequate Battery: yes (X), no (); Peer review: yes (), no (X)]

- Minimal mononuclear cell infiltration of vagina in 4/10 (♀ HD-3 mos).
- Minimal follicular cell hypertrophy of thyroid in 2/10 (♂ HD-3 mos) & 3/20 (♂ HD-6 mos) versus 1/20 (control)
- Minimal necrotic muscle fiber of heart in 3/20 (♂ HD-6 mos) versus 1/20 (control)
- Minimal pigmentation of popliteal lymph node in 17/20 (♂ HD-6 mos) versus 9/20 (control)

- Minimal chronic inflammation of stomach in 6/20 (♂ HD-6 mos) versus 2/20 (control)
- Minimal mixed cell infiltration of trachea in 3/20 (♂ HD-6 mos)
- These observations are not clearly test-article related.

Toxicokinetics:

Day	50 mg eq./kg/day						150 mg eq./kg/day					
	M			F			M			F		
	0	90	181	0	90	181	0	90	181	0	90	181
C _{max} (ng/ml)	2050	1640	2110	2440	3080	2390	3580	3730	3650	5180	4340	4760
AUC ¹⁾ (ng.h/ml)	13000 ²⁾	9450	8320	13800 ²⁾	11700	12700	32900	22500	20900	37100	25600	32800

¹⁾ AUC_{0-∞} (SD) or AUC_{0-24h} (RD)

²⁾ AUC_{0-8h}

Day	500 mg eq./kg/day					
	M			F		
	0	90	181	0	90	181
C _{max} (ng/ml)	5150	3210	4080	5700	5750	7220
AUC ¹⁾ (ng.h/ml)	41300	30200	30200	62800	46400	52700

Table from sponsor

Mortality observations for individual animals (study # NC179):

- Vehicle male No. 17 was sacrificed on day 77. The focal acute pleuritis was considered indicative for a gavage-related death. The atrophy of some lymphoid tissues and of the parotid salivary gland and the single cell death within the Harderian glands were considered secondary to the animal's poor condition.
- Vehicle male No. 25 died on day 93. A gavage accident was considered the most likely cause of death, based on the marked lung congestion with presence of blood and granulocytes in the tracheal lumen and the absence of other relevant findings.
- Vehicle male No. 29 died on day 90. A gavage accident could be concluded from the subcutaneous pyogranulomatous inflammation with central necrosis and foreign particles. The thymic atrophy was secondary to the animals' poor condition. The prominent granulocytes/myelopoiesis in the bone marrow was related to the subcutaneous inflammation.
- Vehicle female No. 222 died on day 108. Only a limited number of tissues could be examined due to cannibalism and the cause of death could not be clearly determined. Based on the moderate lung congestion with minimal edema, the cause of death was possibly gavage-related.
- Female No. 232 (LD) died on day 62. The moderate to marked lung congestion in the absence of other relevant findings was considered indicative of a gavage accident.
- Female No. 257 (LD) died on day 131. The minimal acute and granulomatous inflammation in the lung (with pleuritis) and the acute inflammation adjacent to the thymus were indicative for a gavage related death. The acute/fibrinous tracheitis was

indicative for entry of formulation into the respiratory tract. The centrilobular necrosis in the liver was considered secondary to hypoxia (possibly related to agony).

- Male No. 70 (MD) was killed on day 49. The atrophy of several lymphoid tissues and of the parotid salivary gland and the single cell death within the Harderian glands were considered secondary to the animal's poor condition. The cause of the animal's poor condition was probably related to the gavage procedure as indicated by the minimal focal

acute inflammation at the heart base.

- Male No. 74 (MD) died on day 61. A gavage-related death could be concluded from the marked lung inflammation and congestion and the inflammation adjacent to the larynx and the thyroid gland. The inflammation and focal ulceration in the larynx are indicative for an irritant effect of the formulation.

- Male No. 85 (MD) died on day 68 (cannibalised). A gavage accident was considered the most likely cause of death based on the marked lung congestion.

- Female No. 268 (MD) died on day 44. A gavage related death was concluded based on the acute inflammatory changes in the respiratory tract (larynx, trachea, lungs).

- Female No. 270 dosed at 150 mg eq./kg/day was found dead on day 46. A gavage accident was diagnosed at necropsy (perforation of the esophagus) and histology.

- Female No. 285 (MD) was found dead on day 36 (cannibalised). Inflammation/irritation of the respiratory tract due to entry of formulation (by gavage or aspiration) was considered the cause of death. This was concluded from the moderate acute laryngitis and the marked acute, fibrinous tracheitis with ulceration.

- Male No. 117 (HD) died on day 171. The subcutaneous subacute/necrotizing inflammation (with cyst formation and foreign content) and the acute inflammation in the lungs and at the heart base and pericardium, were indicative for a gavage accident. The extramedullary hematopoiesis was related to the inflammatory changes. The thymic atrophy was secondary to the animal's poor condition.

- Female No. 291 (HD) was found dead on day 36. A gavage accident was concluded based on the marked tracheitis, moderate lung congestion and minimal acute inflammation in the lungs and adjacent to the larynx and thyroid.

- Female No. 320 (HD) died on day 102. The acute/fibrinous tracheitis was indicative for entry of formulation into the respiratory tract.

Study title:	<u>2-wk oral (gavage) toxicity study in beagle dogs</u>
Study #:	NC161
CTD location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 19, 2006
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	TMC435350, ZR494617PFA021, 95.5%

Key findings: The NOAEL is 10 mg/kg/day based primarily on 1) changes in urine characteristics including the presence of moderate to high levels of bilirubin, presence of blood, high turbidity and changes in color, 2) a moderate reduction in cholesterol and increases in total and direct bilirubin, 3) a reduction in reticulocytes, and 4) some gross and histopathological changes observed at the mid and high doses.

Methods:

Doses:	0,10,40,(120)[160] mg/kg/day (♀)[♂]
Species/strain:	Beagle dogs
#/sex/group (main study):	3
Route/formulation/volume/infusion rate:	oral gavage/PEG400, 2.5% Vit E-TPGS, NaOH/2 ml/kg/day/NA
#/sex/group (satellite for TK or recovery):	none
Age:	~8 mos
Weight:	7.6 to 10.5 kg(♂); 6.2 to 7.9 kg (♀)
Sampling times:	1&14d(TK);1&14d (Hem/ Coag/ Urin); -1,7,14d CC

Results:

Mortality (2x/day): None due to test article. Mortality observed in 3/24 animals between day 11 & 13, thought to be due to aspiration of formulation into lungs (1♂&2♀; 2 MD & 1 HD).

Clinical signs (2x/day): Did not include observations due to formula aspiration.

- Dog with hypoactivity, reddish colored vomit (3x), abdominal breathing/tachypnea occurring 1 to 5 hrs after dosing on day 11, consist with pathology finding on day 15 of aspiration bronchopneumonia (also food consumption 50-75% & 7%↓ BW of control etc.) (♂LD).
- Slight hypersalivation ↑ 1/2 MD ♂ (once), 1/3 HD ♂(6x), 3/3 HD ♀ (7x).
- Abnormal color/colorless/litter & moderate lameness-right hind limb 1-HD (♂).
- No significant clinical observations: 2/2 LD, 1/2 MD & 1/3 HD (♂); 3/3 LD, 2/2 MD & 0/2 HD (♀).

Body weights (2x/wk): 9-12%↓ in 1/3 HD ♀&♂ (2-3%↓ in controls).

Food consumption (1x/day): Animals w/↓ BW: 50-75% of control (1/3 HD ♂), 50-75% of control (1/3 HD ♀). Other: 50-75% of control [1/3 HD ♀ (5%↓ BW).

Ophthalmoscopy: Performed days: -1 & 13. No test-article related changes noted.

EKG: Performed days: -1, 7 & 13. ↓HR (73+/-15 vs. 130+/-10); ↑QT interval (24+/-0 vs. 19+/-1.2) (HD ♂ 13d). Conclusion: HR ↑ & QT↓ in controls. Values in HD ♂ group are within historical norms.

Hematology:

- % reticulocytes ↓185-270% (HD ♀ & ♂ 13d).
- Leukocytes ↓191% (HD ♀ 13d).

Coagulation: No test-article related changes noted.

Clinical chemistry: (also measured Troponin I & CK)

- Cholesterol ↓40(42-60)-59(~70)% versus pre-dose (control) values (HD ♀&♂ 7&13d).
- Total bilirubin ↑167(267)-300(267)% versus pre-dose (control) values (HD ♀&♂ 13d); ↑187(250)% versus pre-dose (control) values (HD ♂ 7d).
- Direct bilirubin ↑300% versus day 2 values (HD ♀&♂ 13d).
- Total bilirubin ↑67(167)% versus pre-dose (control) values (MD ♀&♂ 13d)

Urinalysis:

- 2/2 HD ♀ had moderate levels of bilirubin and dark yellow color (not present in controls or predose)
- 1/3 HD ♂ had high levels of bilirubin, dark yellow/brown color & moderate presence of blood (no RBC) (not present in controls or predose)
- 1/3 HD ♂ had moderate levels of bilirubin and high turbidity (not present in controls or predose)

Gross pathology:

- Many punctiform red foci, with edema, noted in the fundic area (stomach) with multifocal necrotizing vasculopathy (1/3 HD ♂). Gross findings correlated with histopathology findings: mucosal/submucosal hemorrhage & edema.
- Small thymus with moderate to marked thymus atrophy in 2/2 ♀ & 1/3 ♂ (HD), and in 1/2 ♀ (MD) & 1/3 ♂ (LD).
- Black deposit in bile of gall bladder (2/3 ♂ HD); marked yellow deposit (1/2 ♀ HD).
- Inflammatory cells in portal tracts in liver (slight) in 2/3 ♂ (HD).
- Slight (marked) sinusal hemorrhage of mesenteric (bronchial) lymph node in 2/3 (1/3) ♂ (HD).
- Slight vagina epithelium vacuolation (2/2 ♀ HD).

Organ weights: No test-article related changes noted (see above).

Histopathology: Notes: All study groups evaluated. Heart also evaluated by EM.

[Adequate Battery: yes (X), no () ; Peer review: yes (), no (X)]

- Minimal liver cholestasis noted in 1/3 ♂ & morbid ♀ (HD).
- Observations (1/3 HD ♂): 1) acute endocardial and myocardial necrosis, restricted to LV subendocardial area; 2) a single intramural artery contained eosinophilic material, indicative of fibrinoid degeneration; 3) necrotizing arteriopathy (arterial lesions), affected mid-size arteries from stomach to the rectum, in the tunica submucosa, muscularis and serosa, also in the urinary bladder and in adipose tissue surrounding the salivary glands. Lesions were at

different stages and characterized by intimal and medial fibrinoid necrosis, proteinaceous leakage, and subsequent neutrophilic leukocytoclastic vasculitis.

- Small focus of acute myocardial necrosis, in LV papillary muscle in 1/2 ♀ (MD).
- Several randomly distributed hepatocellular necrotic foci (minimal necrosis) noted in 2/3 ♂ (HD). Foci consisted of pale eosinophilic hepatocytes, whose outline was often still preserved, with neutrophils and extravasated erythrocytes.

Toxicokinetics: Liver & heart/plasma ratios from 4.4 to 66 & 1 to 2 (all dose groups ♂ & ♀).

TMC435350 Dose (mg/kg/day)	Day of Sampling	C _{max} (µg/mL)		AUC ^a (µg.h/mL)	
		M	F	M	F
10	1	12.8	15.4	67.2	83.8
	14	13.8	14.3	73.3	72.4
40	1	21.4	15.7	434	120
	14	71.9	36.5	876	520
160 (M)	1	42.8	66.7	645 ^b	1310 ^b
120 (F)	14	81.2	93.3	1510	1660

^a: AUC_{0-∞} after single dose and AUC_{0-24h} after repeated dose; ^b: AUC_{0-24h}

Table from sponsor

Study title:	<u>1-mos Repeated Dose Oral Toxicity Study of TMC435350 in Beagle Dog w/ 1-mos Recovery Period.</u>
Study #:	NC175
CTD location:	4.2.3.2
Conducting laboratory and location:	Johnson and Johnson Pharmaceutical Research & Development, Global Preclinical Development Beerse site
Date of study initiation:	December 20, 2006
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	TMC435350, ZR494617PFA031, 94.9%

Key findings: The NOAEL is 10 mg/kg/day based primarily on 1) clinical observations including reductions in body weight and food consumption and increased ptyalism and mucoid/hemorrhagic feces, 2) clinical chemistry changes including moderate reductions in cholesterol and total protein and increases in total and direct bilirubin and ALT, 3) hematological changes including a reduction in reticulocytes correlating with small reductions in RBC, hemoglobin and hematocrit and 4) some gross and histopathological changes including minimal to slight multi-focal hepatocyte necrosis observed at the mid and high doses. No test-article related changes were noted in the 1 month recovery group except ALT (92%) and urine color (67-100%) were still increased in female and male and female dogs, respectively.

Methods:

Doses:	0, 10, 30, 90 mg/kg/day
Species/strain:	Beagle dogs
#/sex/group (main study):	3
Route/formulation/volume/infusion rate:	oral gavage/PEG400/1 ml/kg/day/NA
#/sex/group (satellite for TK or recovery):	2-2 groups (recovery)
Age:	6.5 to 8 mos
Weight:	5.8 to 9.8 kg
Sampling times:	1&27d(TK);-7,14,28d (42&56-recovery) (Hem/Coag/Urin/CC)

Results:

Mortality (1x/day): None. Note: Observations also made 1, 2, 4, 6 & 24 h post-dosing (1x/wk).

Clinical signs (1x/day): Note: Observations made 1, 2, 4, 6 & 24 h after dosing (1x/wk).

- Slight hypersalivation ↑ 5/5 HD ♂, 4/5 HD ♀ (versus 2/5 in controls).
- Mucoïd 2/3 ♂ & 1/3 ♀ (MD), 3/5 (♂&♀ HD) and hemorrhagic 1/5 ♂ (HD) feces.
- No test-article related changes noted during recovery period.

Body weights (1x/wk):

- 5-7%↓ in ♀ (MD) & ♀&♂ (HD) versus controls (28d) (%↓ in ♀ evident at 21d).
- Weight gain in HD group comparable to control during recovery period.

Food consumption(1x/wk):

- 25-31%↓ in HD ♀ versus control (7d through 28d).
- HD ♀ consumption comparable to control during recovery period.

Ophthalmoscopy: Performed days: -7, 14, 24, 55 (recovery). Distichiasis & conjunctival hyperemia observed in 1/5 ♂ (HD) starting at 2 wks. May not be test-article related-common in historical controls.

EKG: Performed days: -8, 13, 27(2-2.5h post-dose), 55 (recovery). No test-article related changes noted.

Hematology:

- ↓% (total) reticulocytes 27-43% (31-50%) (HD ♂ & ♀ 14d) versus control.
- ↓% (total) reticulocytes 14-27% (29%) (MD ♀ & ♂ 28d); 27-43% (32-45%) (HD ♂ & ♀ 28d) versus control.
- ↓ in total RBC, hemoglobin & hematocrit: 5-7% (MD ♀ 28d), 6-8% (HD ♂ 28d) & 5-9% (HD ♀ 28d) versus control.
- Recovery period: ↓ in total RBC, hemoglobin & hematocrit: 8-12% (HD ♀ 42d) versus control, and all fully recovered at 56d (MD & HD ♀ & HD ♂).

- Recovery period: (rebound) ↑% & total reticulocytes at 42d versus control (27-29% in HD ♀ & 93-100% in HD ♂); still ↑ at 56d in HD ♀ (78-83%) but other groups at control levels (MD ♀ & HD ♂).

Coagulation: No test-article related changes noted.

Clinical chemistry: (also measured Troponin I & vWF)

- Total protein (albumin) ↓ 7-10% (10-13%) versus control (♂&♀ HD, 14&28d).
- Cholesterol ↓ at 14&28d versus control: 17-23% (MD ♀&♂), 31-32% (HD ♂) & 43-48% (HD ♀).
- Total bilirubin ↑ at 14&28d versus control: 30-36% (MD ♀), 275% (HD ♂ 14d), 83% (HD ♂ 28d), & 246-250% (HD ♀ 14&28d).
- Direct bilirubin ↑ 550-700% versus control (HD ♀ 14&28d) & values of 0.04 & 0.06 versus control (0.00) (HD ♂ 14&28d).
- ALT ↑ at 14&28d versus control: 74-241% (MD ♀), 20-90% (HD ♂), & 71-381% (HD ♀).
- Recovery period: total bilirubin ↑ 225% versus control (HD ♂ 42d) & ALT ↑ 59-92% versus control (HD ♀ 42&56d). All values at control levels except ALT in HD ♀ at 56d. Recovery in HD ♀??

Urinalysis: Bilirubin not examined.

- Color ↑ 56-214% at 14d & 27-86% at 28d (♂&♀ HD).
- Recovery period: color ↑ 67-100% at 56d (♂&♀ HD). Not recovered?

Gross pathology:

- Slight emaciation 1/3 (♂ MD&HD).
- Aplasia and malformation in epididymides 1/3 (♂ HD).
- Swollen liver (♂ HD).
- Focal/area red on lung 1/3 (♀ HD).

Organ weights:

- Adrenal gland & thymus 19-36%↑ & 29-38%↓ (abs&%) (♂ HD).
- Adrenal gland & thymus (♂ HD) not different than controls (56d).
- Liver 36%↑ (abs) in 1/3 (♀ HD).

Histopathology: All study groups evaluated. [Adequate Battery: yes (X), no (); Peer review: yes (), no (X)]

- Minimal focal hepatocyte necrosis 1/3 (♂ HD).
- Minimal (2/3 MD ♀) to slight (1/3 HD ♂&♀) multi-focal hepatocyte necrosis.
- Minimal involution of thymus (1/3 HD ♀).
- Minimal mucosal hyperplasia of gall bladder (1/3 ♂ HD).
- No test-article related changes noted in recovery animals.

Toxicokinetics:

	Male			Female		
	Day 0					
Dose (mg eq./kg/day)	10	30	90	10	30	90
C _{max} (ng/ml)	9425	28130	27100	4000	34900	101150
AUC _{0-inf} (ng.h/ml)	32860	202595	437284 ¹	16666	267695	1441836 ¹
	Day 27					
	Dose (mg eq./kg/day)	10	30	90	10	30
C _{max} (ng/ml)	4015	29500	93450	4565	40450	94750
AUC _{0-24 h} (ng.h/ml)	15549	201333	1477769	18581	374131	1709919

¹ AUC_{0-24 h}

Table from sponsor

Study title:	<u>TK and escalating IV and oral (by gavage) dose range finding study followed by an oral 14- or 28-day fixed dose phase in the male rhesus monkey</u>
Study #:	NC181
CTD location:	4 2 3 1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 17, 2006
GLP compliance:	No
QA report:	No
Drug, lot #, and % purity:	TMC435350, ZR494617PFA021 or LVDE_0162_026_1 and JNJ 38733214-AAA, 98.2% or 96.9%

Key findings: No NOAEL determined due to study design lacking more than one treatment group. Toxicity observations included: feces discoloration (orange/red/dark red/brown) with the presence of blood on only one occasion, slight reductions in RBC, HCT & PCV%, increases in total and direct bilirubin and AST, a decrease in cholesterol, traces of blood detected in urine, and some histopathology changes. Toxicity observations in 14-day repeat dose (20 mg/kg/day) animals were limited to hypersalivation, emesis, and increased AST.

Methods:

Doses:	See below
Species/strain:	Rhesus monkeys
#/sex/group (main study):	8 ♂ only. 2 animals used for MTD portion
Route/formulation/volume/infusion rate:	IV or oral gavage/PEG400, 2.5% Vit E-TPGS, NaOH/see below
#/sex/group (satellite for TK or recovery):	none
Age:	6 to 10 yrs
Weight:	5.8 to 13 kg

Sampling times:	MTD phase: pre-dose, 2,6,10,16&20h post-dose; Fixed dose phase 1&2: pre-dose, 1&7d or biweekly (Hem/Coag/Urin/CC)
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Maximum tolerated dose (MTD) phase

Group number	Group description	Color code	Dose level (mg/kg/day)	Day of study	Number of animals Males	Application volume (mL/kg)
1	Vehicle (orally)	Red	0	1	2	1
	Low dose (intravenously)	Red	5	5	same	2.5
	Low dose (orally)	Red	20	9	same	1
	Medium dose (orally)	Red	150	15	same	1
	High dose (orally)	Red	300	19	same	1

Fixed dose phase 1

Group number	Group description	Color code	Dose level (mg/kg/day)	Day of study	Number of animals Males	Application volume (mL/kg/day)
1	Vehicle	White	0	1-3	2	1
2	High dose (used animals)	Green	200	1-3	2*(a)	1
3	High dose (naïve animals)	Red	200	1-3	4	1

* from dose range finding phase of the study

(a) Animal 11867M was terminated in moribund condition after dosing on day 1 of the study

Vehicle test phase

Group number	Group description	Color code	Dose level (mg/kg/day)	Day of study	Number of animals Males	Application volume (mL/kg/day)
1	Vehicle (formerly group 1 animals)	White	0	1-4	2	1
2	Vehicle (formerly group 3 animals)	Red	0	1-4	2	1

Fixed dose phase 2 (20 mg/kg/day)

Group number	Group description	Color code	Dose level (mg/kg/day)	Day of study	Number of animals Males	Application volume (mL/kg/day)
1	Vehicle	White	0	1-15	2	0.25
2	High dose	Red	20	1-15	5	0.25

Fixed dose phase 2 (60 mg/kg/day)

Group number	Group description	Color code	Dose level (mg/kg/day)	Day of study	Number of animals Males	Application volume (mL/kg/day)
1	Vehicle	White	0	16-43	2	0.25
2	High dose	Red	60	16-43	3	0.25

Tables from sponsor

Results:

Mortality (1x/day): None due to test article. Mortality observed in 2/8 animals, due to aspiration of formulation into lungs (1st was day 1 of “fixed dose phase 1” part and the 2nd was identified at necropsy of “fixed dose phase 2 (20mg/kg/day)” part). PK analysis indicated ↑ amount of TMC435350 in lungs compared to other animals. The 1st animal had severe lung lesions (necrotizing bronchiolitis & hemorrhagic bronchopneumonia) while the 2nd animal had reddish lungs w/ dark/firm area in left caudal lobe, multifocal bronchopneumonia (partly granulomatous w/ foreign material) and pleuritis (w/ few giant cells and grey foreign material).

Clinical signs (1x/day):

- For fixed dose phase 1 part: dosing discontinued due to feces discoloration (orange/red/dark red/brown) on 4d without other adverse clinical signs that persisted in all animals until 7d. The presence of blood in these fecal samples was excluded except for one occasion on 5d. For vehicle test phase, possible GI intolerance and systemic toxicity of the vehicle was ruled out by oral gavage for 4d.
- For fixed dose phase 2 part (20 mg/kg/day): slight 5/5 to severe (1/5) hypersalivation and emesis (1/5).

Body weights (1x/wk): No test-article related changes noted.

Food consumption: No test-article related changes noted.

Ophthalmoscopy: Not performed.

EKG: Also BP predose and 3h post-dose. No test-article related changes noted.

Hematology:

- For fixed dose phase 1 part: slight ↓ in RBC & in PCV% (11-13% compared to pre-dose & control).
- For fixed dose phase 2 (60 mg/kg/day) part: slight ↓ in RBC, HCT & PCV% (~9% compared to control-28d).

Coagulation: No test-article related changes noted.

Clinical chemistry: (also measured Troponin I, myoglobin & CK)

- For MTD part: ↑ ~250% in total bilirubin, ↑ ~190-260% in AST & 40%↓ in cholesterol (300 mg/kg).
- For fixed dose phase 1 part (200 mg/kg/day): slight ↑ in total bilirubin (~300% compared to pre-dose), direct bilirubin (~280% compared to controls) & AST

(~320% compared to pre-dose) in treated animals on day 1 (pool of plasma samples from 3, 5 and 8 hours post-dose). An increase in AST (~450% compared to pre-dose) also observed in 1 control animal.

- For fixed dose phase 1 part: total bilirubin remained elevated in 2 treated animals (~350% compared to pre-dose) observed on day 7 (after 3 days of recovery/wash out).
- For fixed dose phase 2 part (20 mg/kg/day): moderate ↑ ~400-450% in AST of 2/5 (one of these animals had signs of formulation in the lungs-see above).
- For fixed dose phase 2 part (60 mg/kg/day): moderate ↑ in total bilirubin ~40%-13d (2/3) & 330%-28d (3/3), & in direct bilirubin (~70%-13d & 581-737%-28d in 2/3), & 40-46%↓ in cholesterol versus pre-dose and control levels.

Urinalysis:

- For fixed dose phase 1 part: traces of blood detected in 3 treated animals (200 mg/kg) on day 7 (after 3 days of recovery/wash out).
- For fixed dose phase 2 (60 mg/kg/day) part: blood occasionally detected in treated & control animals.

Gross pathology: See histopathology below.

Organ weights: No test-article related changes noted.

Histopathology: All study groups evaluated. Heart also evaluated by EM. [Adequate Battery: yes (X), no ()]; Peer review: yes (), no (X)]

- After fixed dose phase 2 part:
 - ↑ eosinophilic cortical infiltration of adrenals (2/5)
 - ↑ inflammatory cell foci in heart (5/5)
 - ↑ hepatocyte vacuolation (2/5)
 - ↑ subacute prostate inflammation (2/5)
 - ↑ subacute stomach inflammation (4/5)

Toxicokinetics:

Table I: Mean C_{max} , AUC and $t_{1/2}$ values of TMC435350 during the MTD phase

Parameter		20 mg/kg/day	150 mg/kg/day	300 mg/kg/day
C_{max}	ng/mL	10065	30850	56700
NC_{max}		503.3	205.7	189.0
AUC_r	ng.h/mL	41974	253204	822365
$NAUC_r$		2099	1688	2741
AUC_{∞}	ng.h/mL	42463#	261760	2691997#
$NAUC_{\infty}$		2123#	1745	8973#
$t_{1/2}$	h	3.884#	4.159	24.52#

#: accurate determination not possible

Fixed Dose part of the study

		Day 1: 200 mg/kg/day (n=5)	Day 1: 20 mg/kg/day (n=5)	Day 14: 20 mg/kg/day (n=5)	Day 22 (7): 60 mg/kg/day (n=3)	Day 37 (22): 60 mg/kg/day (n=3)
Parameter		Mean ^{a)} ± SD	Mean ^{a)} ± SD	Mean ^{a)} ± SD	Mean ^{a)} ± SD	Mean ^{a)} ± SD
Dose	mg/kg	200	20	20	60	60
C _{max}	ng/mL	55500 ± 19846	7318 ± 6311	8830 ± 9584	40000 ± 30848	40400 ± 10096
NC _{inax}		277.5 ± 99.2	365.9 ± 315.5	441.5 ± 479.2	666.7 ± 514.1	673.3 ± 168.3
t _{max}	h	8.0(8.0-8.0)	5.0(3.0-8.0)	5.0(2.0-8.0)	5.0(3.0-8.0)	5.0(5.0-8.0)
t _{last}	h	24.0(24.0-24.0)	24.0(24.0-24.0)	24.0(24.0-24.0)	24.0(24.0-24.0)	24.0(24.0-24.0)
AUC _{last} =AUC _t	ng.h/mL	827866 ± 269592	52286 ± 45228	85238 ± 120085	749721 ± 617664	662719 ± 278525
NAUC _{last} =NAUC _t		4139 ± 1348	2614 ± 2261	4262 ± 6004	12495 ± 10294	11045 ± 4642
AUC _∞	ng.h/mL	2031786# ± 1270162	53482# ± 45817	-	-	-
NAUC _∞		10159# ± 6351#	2674# ± 2291	-	-	-
λ _z	1/h	0.04510# ± 0.02718	0.1706# ± 0.0183	0.1451# ± 0.0269	0.05898# ± 0.05519	0.06534# ± 0.04368
t _{1/2}	h	21.11# ± 14.00	4.101# ± 0.434	4.925# ± 0.995	19.40# ± 12.77	15.61# ± 12.14

a) t_{max}, t_{last}: median value (range)
 -: not calculated
 #: accurate determination not possible

Tables from sponsor

Repeat-dose toxicity (other):

- 13-26 wk oral beagle dog study (NC178, 0, 5, 15, 60 mg/kg/day, n=3/sex/group, Research lab: (b) (4) was terminated because of mortality observed in 3 animals on day 1 & 2, due to aspiration of formulation into lungs (3♂; 2 MD & 1 HD), in the absence of test-article related changes noted in all other animals.

APPEARS THIS WAY ON ORIGINAL

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/s/

JANICE A LANSITA
08/22/2013

HANAN N GHANTOUS
08/22/2013

Comments on NDA 205123 simepravir

From A. Jacobs, AD

Date: 8/21/13

1. I concur that there are no pharm-tox approvability issues.
2. I concur with the reviewer that the pregnancy category should be C and some special studies listed by the applicant in proposed labeling under animal toxicology studies should be deleted.
3. I have conveyed some editorial suggestions to the reviewer and they will be addressed as appropriate

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/s/

ABIGAIL C JACOBS
08/21/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 205123

Applicant: Janssen

Stamp Date: March 28, 2013

**Drug Name: Simeprevir/TMC435 NDA Type: NME and New
Combination**

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	The sponsor did not describe teratogenic findings observed in a pilot embryofetal mouse study that included exencephaly and protruding tongue. This issue will be discussed with the sponsor and addressed during the labeling review.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			NA
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? YES

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Not applicable. The NDA is fileable.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None.

Janice Lansita 4/29/13

 Reviewing Pharmacologist Date

Hanan Ghantous 4/29/13

 Team Leader/Supervisor Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JANICE A LANSITA
04/29/2013

HANAN N GHANTOUS
04/29/2013